JOURNAL

OF THE

AMERICAN SOCIETY

OF

SUGAR BEET TECHNOLOGISTS

VOL. IX, NO. 3 OCTOBER, 1956

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JOURNAL
of the
American Society of Sugar Beet Technologists

Volume IX
Number 3
October, 1956

Published quarterly by

The American Society of Sugar Beet Technologists
Office of the Secretary
P. O. Box 538
Fort Collins, Colorado, U. S. A.

Subscription prices:

$4.50 per year, domestic
$5.00 per year, foreign
$1.25 per copy, domestic
$1.40 per copy, foreign

Made in the United States of America

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Distribution, Relation to Weeds, and Histology of Sugar Beet Root Galls Caused by Nacobbus Batatiformis Thorne and Schuster[1]

M. L. Schuster and Gerald Thorne[2]

Galled roots from sugar beets and other economic crop plants collected in western Nebraska in 1949 contained nematodes which differed from the commonly recognized root knot nematodes (*Meloidogyne* spp.) of economic plants. Thorne and Schuster (2)[3] identified and named the nematode *Nacobbus batatiformis* Thorne and Schuster, 1956. The present paper includes investigations concerning the distribution, mode of dissemination, relation of weeds, and the histology of the galled roots of sugar beet caused by the newly described nematode.

Distribution in Western Nebraska

The pattern of distribution of the new nematode in the western Nebraska panhandle became apparent as a result of a survey in 1953 and 1954, in Scotts Bluff, Sioux, and Morrill counties. Included in this survey were 125 sugar beet fields selected at random: 71 in 1953 and 54 in 1954. *Nacobbus batatiformis* was found in 32 percent of the fields. This new nematode appeared in combination with *Heterodera schachtii* Schmidt in one percent of the fields. *Meloidogyne hapla* Chitwood appeared in 16 percent of the samples, in 12 percent in combination with the new nematode. Although several species of *Meloidogyne* are described, it was deduced from previous studies that the northern root knot nematode, *M. hapla,* was present in western Nebraska.

Sampling in August or September insured the presence of mature nematodes in infected roots, which aided in genus determination. Representative root samples stained in hot lactophenol acid fuchsin mixture aided in locating the nematodes to be identified.

The survey shows *N. batatiformis* to be present north of the North Platte River but not south of it where the soil of examined fields is primarily silt loam. The soil class north of the river is sandy loam or very fine sandy loam. The distribution pattern of the new species may be coincidental to soil class. Neverthless, this conforms to findings that *Meloidogyne* is more common on coarse-textured than on fine-textured soils. It might be stated that although *M. hapla* was found in 16 fields it is not of much economic importance on sugar beets; on occasions it disqualifies seed potatoes for certification by its presence in the tubers. *Nacobbus batatiformis,* on the other hand, is important economically on sugar beets causing reduced stands and lower plant vigor.

Relation of Native Plants to New Nematode

The fact that *N. batatiformis,* which produces such very obvious symptoms on sugar beets and other plants, was not found elsewhere indicated

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[1] Published with the approval of the Director, Paper No. 769, Journal Series, Nebraska Agricultural Experiment Station.

[2] Associate Plant Pathologist, Department of Plant Pathology, Nebraska Agricultural Experiment Station, Lincoln, Nebraska, and Senior Nematologist, Section of Nematology, Agricultural Research Service, United States Department of Agriculture, Salt Lake City, Utah.

[3] Numbers in parentheses refer to literature cited.

Research funds for this study were contributed in part by the Great Western Sugar Company, Denver, Colorado.

that it might be native to western Nebraska. Native weeds on virgin or uncultivated land were checked for galls and for the associated nematodes. Three species of cacti infected with *N. batatiformis* were located on cattle ranges or uncultivated areas near Henry and Mitchell, and along a main irrigation canal north of Mitchell in an area which abuts a cattle range. These infected cacti were *Opuntia tortispina* Nutt., *O. fragilis* (Nutt.) Haw. and *Coryphantha vivipara Nutt.*[4] (Figure 1).

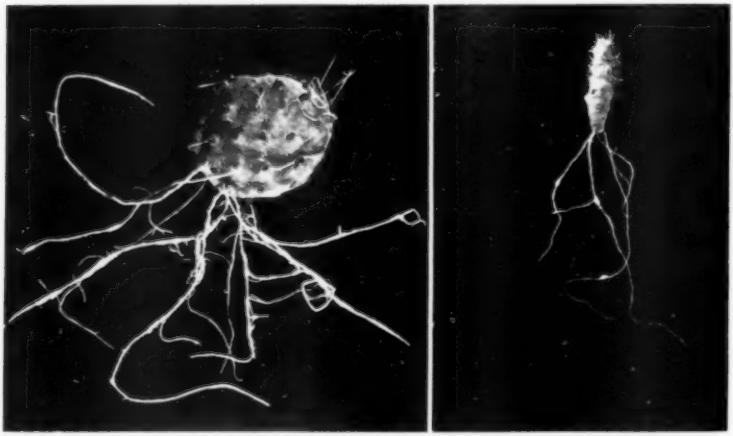

Figure 1.—Cacti native to western Nebraska with root galls caused by *Nacobbus batatiformis*. Left, *Opuntia tortispina*. Right, *Opuntia fragilis*.

Experiments in 1954 and 1955 with the new species of nematode emphasized the similarity of isolates from sugar beets and cacti. Cross inoculation tests with the isolates indicated that the nematodes on cacti and sugar beets were similar since reciprocal infection resulted. The progeny from sugar beets were from a single egg sac: the isolates from the cacti consisted of a population transfer. Each inoculation consisted of four replications. The morphology and mode of reproduction of the isolates from sugar beets and cacti were similar. On the basis of this information it is assumed that *N. batatiformis* is indigenous to western Nebraska. Another important point in justifying this assumption is that the organism is capable of reproducing in naturally infected cacti, as well as in artificially infected plants.

Relation of Weeds to Control by Crop Rotation

An explanation for occasional ineffective control by rotation with nonsusceptible crops included the possible dissemination of nematodes in irrigation water and the importance of weeds as harborers of the pathogens. Weeds investigated from this standpoint were *Kochia scoparia* (L.) Schrader and *Chenopodium album* L. in roots of which *N. batatiformis* causes pronounced galling and completes its life cycle.

[4] Dr. J. F. Davidson, Curator of Botanical Herbarium-Museum, University of Nebraska, stated that cacti deposited in the herbarium include these three species.

Kochia scoparia appears most important from the standpoint of harboring the nematode in and along irrigation ditches in western Nebraska. This weed commonly grows in an area north of Mitchell where the root knot disease is most important. These infected plants were found the entire distance from a main canal (Interstate) and the Dry Spottedtail Creek to the ditches which supply water to individual farms. The area most intensively studied included parts of sections 16, 17, 20, and 21 of T-23-N and R-56-W. This information is circumstantial evidence that *N. batatiformis* is spread by irrigation water.

Attempts to determine spread of root knot nematodes by isolation from irrigation water proved incomplete. It was found that many nematodes could be obtained by screening water from an irrigation ditch in and along which were present infected *Kochia*. In two separate tests in 1955, 1073 and 298 nematodes were isolated from irrigation water conducted through a one-inch siphon tube and were deposited on a 300 mesh soil screen in one hour. Although only two nematodes among those isolated appeared to be larvae of *Nacobbus batatiformis*, this test emphasizes the importance of water in the movement of these organisms. The finding of greater populations of the root-knot nematode may involve proper timing and sampling.

Ineffective control, even when proper crop rotation is practiced, could conceivably be caused by the persistence of the weeds, *Kochia scoparia* and *Chenopodium album* in the cultivated fields, acting as hosts. Host range studies have shown that the main crop plants, except sugar beets grown under irrigation, were not susceptible to *N. batatiformis* (2). This nonsusceptible group included potatoes, beans, corn, barley, oats, alfalfa, and sweet clover. Two fields near Mitchell proved suitable areas for investigation. One field in alfalfa for the past five years contained scattered infected *Kochia scoparia* and *Chenopodium album*. Another field in barley-alfala, which was in sugar beets the previous year, contained many severely infected *Kochia scoparia* plants. Thus it appears that weeds in fields of non-susceptible crops and in irrigation ditches serve as harborers of the organism and under these circumstances nullify the purpose of crop rotation from the standpoint of root knot control.

Pathological Histology

After penetration of the root, the larvae of *N. batatiiformis* usually orient themselves near the vascular tissue. The first visible disease symptoms are a necrosis of cell walls and increased cell division in the vicinity of the nematode. These effects are apparent when the larvae assume a spiral or "C" form commonly found in the early stages of infection.

Gall formation is the result of increase in cell size and numbers. Comparison of histological symptoms of advanced stages of gall development caused by *N. batatiformis* with those caused by *Meloidogyne* spp. indicated differences and similarities. *Meloidogyne* usually produces in each gall several, usually three to six, giant cells, that remain as distinct units (1). Comparable to these giant cells in galls produced by *N. batatiformis* is a more extensive area near the *N. batatiformis* oral region which usually assumes a spindle or egg shape with the female's head imbedded in one end (Figure 2). The "spindle" may be distorted in shape depending on the assymetry

of the root; it is localized in a definite area delimited possibly by the endodermis (Figure 3). The long axis of the "spindle" is most commonly parallel to the main axis of the root.

The "spindle" appears to function as a unit. The individual cells may lose their identity in advanced stages and the entire area may be of an amorphous tissue type with cell walls that do not take on the characteristic

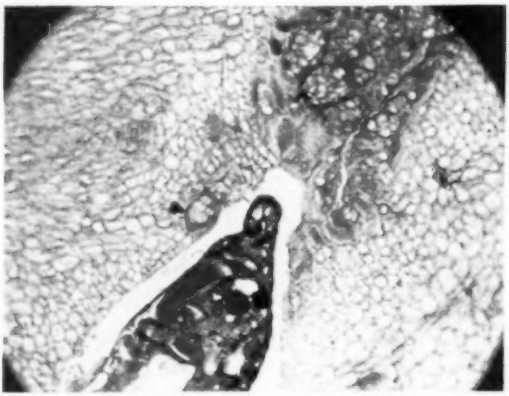

Figure 2.—Longitudinal section of a portion of sugar beet root infected with *N. batatiformis* showing anterior portion of female and effect on root. Observe presence of numerous globules (probably fat or oil in nature) in cells near head of nematode and the apparent dissolution of cell walls in this area. Photomicrograph. About 144X.

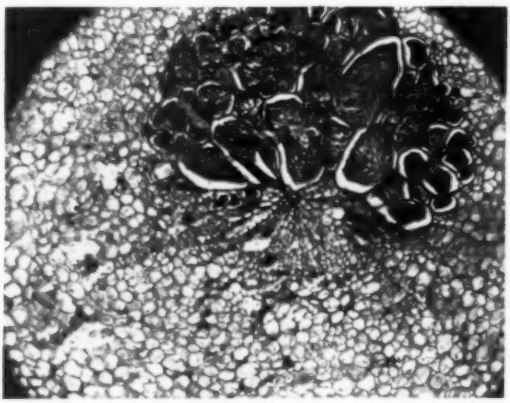

Figure 3.—Transection of a portion of sugar beet root infected with *N. batatiformis*. Observe the extensive area that stains differently because of the presence of the nematode (which is not evident in this photograph) and is limited to the stelar area of the note; note hypertrophied cells. Photomicrograph. About 144X.

stain of normal walls. Cell walls commonly collapse, and enlarged cells result from "coalescence" of adjacent cells; cell enlargement also results from accentuated cell growth (Figure 3). Nuclei occasionally divide without concomitant cell division and multinucleate cells result.

In some histological sections of certain galls, walls of the "spindle" cells appear thickened with deeply staining areas occurring in the "corners" of the cells. These effects may be due to a breakdown of cell walls. Since all the cells of the spindle possess thickened walls these results could not be due to transformation of undifferentiated xylem elements.

Unstained, the "spindle" area appears grayish or granular and can be readily differentiated from the remainder of the root tissues. In stained sections the "spindle" area appears distinct because of differences in staining reaction when a combination fast green-safranin or fast green-acid fuchsin stain or fast green alone is employed. In stained sections, the cells contain either a fibrillar or globular materials. It is assumed that these cytoplasmic materials give the unstained tissue the granular appearance.

The effect on the root is similar to that of *Meloidogyne* spp. in that infection seems to cause a movement of nutritive material into a region about the nematode's oral region as evidenced by the rich granular cytoplasmic contents in the "spindle" area. This localization of materials and their removal from the plant's use undoubtedly affects the metabolism and growth of the plant.

Infection outside the vascular cylinder results in galls, or "warts," on the surface of older tap roots, arising almost entirely from cortical tissue. A structure similar to the vascular spindle is formed, but it is not sharply delimited and is more irregular in shape. It is near the proximal region of the nematode, however, and takes the same stain as the vascular spindle. Cell wall thickenings have not been observed in these instances, however.

Summary

Nacobbus batatiformis was found in 32 percent of sugar beet fields examined in western Nebraska.

Native cacti appear to be important hosts and therefore sources of the new nematode; it is therefore assumed that *N. batatiformis* is indigenous to the state.

Weeds play an important role in the harboring of the nematode in fields and irrigation ditch banks. Circumstantial evidence indicates that irrigation water is a vehicle for transport of root knot nematodes.

Histological studies of *N. batatiformis*-produced galls indicate differences from and similarities with those caused by *Meloidogyne* spp.

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(1) CHRISTIE, J. R. 1936. The development of root-knot nematode galls. Phytopathology 26:1-22.

(2) THORNE, GERALD and M. L. SCHUSTER. 1956. *Nacobbus batatiformis* n. sp. (Nematoda: Tylenchidae), producing galls on the roots of sugar beets and other plants. Proc. Helminth. Soc. Wash. 23 (2) :/128:134.

Beet-Leaf Silage

E. J. Maynard[1]

One of the more difficult problems in connection with the sugar beet harvest has been the proper harvesting and most efficient conservation of the sugar beet tops for livestock feed. A brief review of some of the attempts made in this direction during the past 40 years should be of interest in connection with a discussion of this latest conservation method which seems to have especially intrigued the popular fancy of many farmers and livestock feeders at the present time.

The pasturing of field-cured, dried beet tops by cattle or sheep has been a common practice throughout the West for many years. Especially in arid climates, field-cured, dried beet tops have been wind-rowed or gathered into small piles in the field in order to help retain sufficient moisture, in such piles, to avoid excessive drying and shattering of the beet leaves. Piling the tops in such a manner avoids trampling by livestock and also keeps the piles accessible in the event of occasional snow storms. In many instances, too, such small piles or windrows have eventually been hauled in from the field and have been stored dry in ricks or stacks adjacent to the feed lot to further reduce surface exposure and where they become more readily accessible for feeding. It is safe to say that, over our entire beet growing area today, the above-mentioned practices in handling beet tops are still, by far, the most common and widespread.

And through the years, too, there have been established some pretty definite values for beet tops handled in such a manner even though it is perfectly obvious that such values can, in the end, only represent average approximations for any feed crop, subject to such vagaries of weather and climate. At any rate, these approximations have given us something to "tie to" in the past, and should be given consideration in evaluating any new harvesting methods suggested for adoption in the future.

For instance: According to results secured in 27 separate feeding tests conducted at Western Agricultural Experiment Stations, the amount of field-cured, dried tops recovered per ton of beets harvested, produced 10 percent of the weight of beets in dry substance and replaced, or was equal in feeding value to, 46 pounds of corn plus 150 pounds of alfalfa hay, or was equivalent to 112.3 pounds of digestible nutrients. These figures then, have made it possible to satisfactorily demonstrate the relatively high feeding value of field-cured, dried tops, that is of course, dried tops which have been given reasonable care at harvest time. But it is also evident that, with such a perishable crop, there are also many chances for heavy nutritive losses to occur with such methods of handling. Consequently, from the start, it has been recognized that some satisfactory system for gathering and siloing the green beet tops would insure a significantly greater recovery and better conservation of their nutritive value.

That this is a fact has been amply demonstrated by tests conducted over a number of years at the Scottsbluff Experiment Station of the Nebraska Agricultural Experiment Station. Exhaustive studies there have proved that

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[1] General Livestock Consultant, The Great Western Sugar Company.

the tops, from beets grown in a manured rotation with alfalfa hay and siloed in a pit silo, have produced, on the average, 1000 pounds of edible beet-top silage per ton of beets harvested. This silage, fed to lambs or cattle in feeding tests conducted there, has proved equal, pound for pound, in feeding value to good corn silage produced from corn yielding 100 bushels per acre. Obviously then, this properly made beet-top silage is significantly higher in feeding value than the field-cured, dried beet tops. By the same nutritive standards, beet-top silage has provided 181 pounds of digestible nutrients per ton of beets harvested, a 61 percent greater return than that recorded for the field-cured, dried tops.

However, it is also a well-recognized fact that, with the nearly universal acceptance of machine harvest of sugar beets, there have been serious drawbacks to the satisfactory recovery of clean beet tops for siloing. Experience has also demonstrated that beet tops, with a high dirt content, produce a very poor quality silage which gives unsatisfactory results as a livestock feed.

Digressing from the immediate topic for a moment, consider the present attitude of beet growers toward their beet tops. It has been observed that, during the late war years and with the abnormally large profits experienced for livestock feeding operations during that period, many beet growers came to believe that the proper place for their beet tops was under ground; that is, plowed under for fertilizer.

This reminds the author of the time some years ago when some eastern packers were out to see, for the first time, the "wonder crop" beet tops, in the Prospect Valley of Colorado. The fall preceding had been extremely stormy and everyone was hustling to get their beet crop out. In the field they visited, most of the tops had been run over by trucks and had been well mashed into the dirt. A few of the tops were dug out to show the eastern packers what beet tops looked like, whereup one of them said, "Do the cattle have to burrow for them?"

During the past two years, however, there seems to have developed a feeling that this "livestock feeding honeymoon" we have been experiencing is over—at least for the present. There is a crying need to pare feeding costs to the quick and that is where beet tops are quite apt to come back into their own again. This observation has been amply demonstrated by the renewed interest and action in putting up beet-leaf silage during these past two seasons.

This rather circuitous route has been taken to deal with the beet-leaf silage subject because there is not sufficient evidence available to give a final stamp of approval to beet-leaf silage. It can be said, however, that there are, at present, enough field tests and experiments in progress to demonstrate, by this next spring, just how efficient this product is going to be. In the Great Western Sugar Company's territory alone, there are over 50 separate and distinct beet-leaf silage feeding operations in progress at present covering the leaves harvested from some 2,000 acres or 34,000 tons of sugar beets. The company is keeping definite records on these operations.

One of the questions involved with the production of beet-leaf silage has been, "What about the crown?" If, according to the Colorado researchers, the leaf contains only 45 percent of the dry substance of the entire top,

even though it does contain 85 to 92 percent of the carotene and 55 percent of the protein, can we afford to sacrifice the rest of the top? This is a real present day challenge to the manufacturers of beet harvesting machinery because it is not believed there is a single beet harvester on the market today that can do a satisfactory job of harvesting clean green tops for silage along with the harvest of the roots. Furthermore, discerning beet growers are no longer interested in picking up dirty beet tops from a windrow for siloing. The fact is that, the calcareous nature of our western soils is a natural deterrent to the normal fermentation required in silage production and, after all, too much dirt is always detrimental in livestock rations. For this reason, many beet growers have already come to like their beet-leaf silage produced from the leaves and petioles cut from 1 to 4 inches above the crown, even though the ultimate recovery of silage fed is nearer ⅓ than ½ the weight of beets harvested. These beet leaves, by the way, produce a silage with an average crude analysis of 82 percent moisture, 3.9 percent ash, 2.7 percent crude protein, 3.3 percent crude fiber, 0.7 percent fat, and 7.6 percent nitrogen free extract.

Beet growers like the increased efficiency of their mechanical beet harvest after the leaves and weeds have been removed by late models of forage harvesters. One operator claims that, with nothing to interfere with a clear view of the beet row, he has saved an additional ½ to ¾ of a ton of beets per acre and, with the feed saved, has more than paid for his entire harvesting operation.

Beet-leaf silage is a very attractive and healthful feed when produced in any kind of a silo, provided that the silo has free drainage, which for the production of any silage, but especially for this, is essential. Beet-leaf silage can definitely become spoiled when there is not adequate drainage. The run-off juice has relatively little value containing only about six percent dry substance. While it is possible to absorb and retain a part of this run-off juice with some absorbent material such as dried pulp, corn fodder, or straw, most beet growers have preferred to silo the straight beet leaves. Bean straw has proved to be a very poor absorbent material. Beet-leaf silage can be produced in piles on top of the ground, but due to its high moisture and low fiber content, it has a tendency to pancake without the benefit of some type of retaining walls.

While beet-leaf silage cannot be fed in quite as heavy amounts as corn silage, due to slightly more laxative tendencies, it is being fed to all classes of ruminants with good results and has proved especially valuable where there is need for additional protein in the ration. The only adverse comment heard to date has been from the colder regions. Some have found that its high moisture content has caused some freezing in the silo and in the feed bunk.

The present consensus would seem to indicate that, until such time as some beet harvesting machinery comes up with a better method for the recovery, field forage harvesters will continue to be used in a separate operation to recover the leaves for siloing just ahead of beet harvest. The crowns will be left in the field to be pastured or plowed under for manure.

The Purines, Pyrimidines and Nucleosides in Beet Diffusion Juice and Molasses

J. B. Stark, T. Jaouni, and G. F. Bailey[1]

As a continuation of our work on composition of sugar beets and sugar beet liquors (1)[2] the presence of purines, pyrimidines, and nucleosides was investigated and in some cases the quantities determined.

Purines, pyrimidines, and nucleosides are nitrogen-containing compounds that fall in the class of "harmful nitrogen" compounds. Purines contain 3 nitrogen atoms in a fused 5 and 6 member ring structure. Pyrimidines contain 2 nitrogen atoms in a 6 member ring. There may also be amino groups, methyl groups, etc. attached to the ring. Nucleosides are compounds of purines or pyrimidines and a sugar such as ribose. These various compounds are of interest because they are the basic units of nucleotides which in turn form nucleic acids and nucleoproteins. Nucleoproteins, which are present in all cells, are distinctive for different species of plants, but are subject to minor modification. It may be the minor modifications in the nucleoproteins that make the difference between resistance and nonresistance to virus attacks. The purines and pyrimidines are also of interest since nucleoside derivatives such as uridine diphosphate glucose may assist in the synthesis of sucrose in the beet (2).

Increased knowledge of these compounds and methods of separating them may be important in breeding studies, in processing, in improving the storage quality of beets, and possibly, as the source of by-products.

Some work has been done on the purines and pyrimidine composition of European beets. Early work is reviewed by Janacek (3). He lists adenine, guanine, hypoxanthine, xanthine, 7-methylxanthine, and carnine present in beet diffusion juice. Carnine is an indefinite compound and may be a mixture or a compound of hypoxanthine and inosine (hypoxanthine nucleoside). Vavruch (4), using paper chromatography with only slight purification of the sugar beet juice, identified adenine, guanine, hypoxanthine, xanthine, 7-methylxanthine, and guanosine (guanine nucleoside). Uracil was thought to be present. No work on the purine-pyrimidine composition of molasses is known to the authors.

Materials and Methods.

Molasses was obtained from Manteca and Betteravia, California. Diffusion juice was obtained from Manteca and beets for press juice near Davis, California. Ion exchange resins used were Dowex-50, a cation exchanger and Dowex-1, an anion exchanger, (Dow Chemical Company) and Permutit A, an anion exchanger, (The Permutit Company). Chromatographic paper 589, Blue Ribbon, Schleicher and Schuell, was used for paper chromatography with the following solvents: For two-dimensional chromatography, isopropyl alcohol, 170 ml., concentrated hydrochloric acid, 41 ml., water to make

[1] Chemists, Western Utilization Research Branch, Agricultural Research Service, United States Department of Agriculture, Albany 10, California.

[2] Numbers in parentheses refer to literature cited.

250 ml. (5) followed by *n*-butyl alcohol saturated with water, 100 ml., concentrated ammonium hydroxide, 1 ml. (6). For one-dimensional chromatography, water-saturated *n*-butyl alcohol (7) and *n*-butyl alcohol, 77 percent water, 13 percent formic acid, 10 percent by volume (7), were used in addition to the other solvents. A Cary Model 11 Spectrophotometer (Applied Physics Corporation) was used to measure the absorbance of solutions in the ultraviolet. A Mineralight Model V-44 (Ultra Violet Products, Incorporated) was used as a source of shortwave ultraviolet light to detect the purine or pyrimidine spots on paper.

Both ion exchange and paper chromatography were used in this Laboratory for the separation and estimation of the purine-pyrimidine type of compounds. Advantage was taken of the strong absorption of ultraviolet light in the region of 260 millimicrons to follow the course of elution and to detect the presence of these compounds on paper. Preliminary work used the general method described by Wall (8). The solution containing purines, etc. was loaded on a column of cation exchange resin (D-50, 60-100 mesh) and eluted with an increasing concentration of hydrochloric acid. To simplify our problem of detection we passed the column eluate through a cell in the Cary Spectrophotometer and obtained a continuous record of the light adsorption at 260 millimicrons. This method was relatively satisfactory for some constituents but compounds that were eluted early were obscured by the presence of large amounts of colored compounds. A different method was necessary to separate the mixture of colored materials from the purines and pyrimidines.

Most of the purines and pyrimidines are adsorbed by either cation or anion exchange resins. A few, such as orotic acid, uracil, and uridine, are too acidic to be adsorbed by cation exchangers and are removed from solution only by anion exchangers. The following method was devised to give a considerable degree of purification prior to paper chromatography. A solution of dilute molasses was passed through a column of cation exchanger (Dowex-50, hydrogen form) and washed with water. The effluent contained acids, sugars, certain purines and pyrimidines such as uracil and some colored materials. This solution was passed through a column of anion exchanger (Permutit A, hydroxide form). The column was washed with water. Sugars and other neutral materials passed through. Acids, pyrimidines, purines, and most colored materials were retained. The column was eluted with saturated aqueous carbon dioxide. The purines or pyrimidines were eluted while the stronger acids and most of the colored material remained on the resin. The solution was concentrated under vacuum. The residue was dissolved in water or dilute (0.1—.5N) hydrochloric acid and chromatographed with a suitable solvent.

Most of the purines remained on the cation resin. These were eluted with 0.4N ammonium hydroxide. The ammonia was removed by vacuum concentration. This solution contained betaine and the amino acids in addition to a number of purines such as adenine and guanine. The solution was passed through a column of anion exchanger (Dowex-1, carbonic acid form). To obtain the resin in this state a suspension of the hydroxide form of the resin was treated with carbon dioxide gas until the pH of the

supernatant was less than 5. With the resin in this form amino acids and betaine pass through and the purines remain and were eluted with aqueous carbon dioxide solution. The resin does not need to be regenerated after each batch and may be used several times. Glutamic and aspartic acids were retained on the resin but were not eluted with carbon dioxide solution. This will give a gradual build-up of these compounds. The purine-containing solution was concentrated under vacuum and chromatographed in a manner similar to the effluent from the Dowex-50.

The purine and pyrimidine spots are located on paper by using a Mineralight having a high light emission at 253.7 millimicrons. Near this wavelength purines and pyrimidines have a high absorbance and show up as purple spots on the paper against a fluorescent background.

For quantitative determinations the original solution of molasses, e.g., 400 ml. of 10 brix molasses, was treated by the ion exchange procedure and concentrated to a small volume. Final concentration and drying may be done in a vacuum desiccator over calcium chloride. There were two samples. The first, called the uracil fraction, is that portion which is not retained on the cation resin. The second, called the adenine fraction, contained those materials that are retained by the cation exchanger. Each is made to a definite volume (10-25 ml.). The samples should be stored in the refrigerator with a small amount of chloroform in the flask. The solutions are applied to the paper with a 0.01-ml. self-filling pipette to a total volume of 0.04 to 0.08 ml. The paper should be dried after each addition. The paper was chromatographed with a suitable solvent and then air dried for several hours. The spots were located with the Mineralight and circled lightly in pencil. They were cut out and extracted for two or more hours in a 10-ml. beaker with 6.00 ml. of 0.1N hydrochloric acid. Approximately 5 ml. of the solution was placed in a 10-cm. micro tube and the absorbance measured in the Cary spectrophotometer. Actually the curve for the absorbance was run from 400 to 220 millimicrons and recorded on the chart. The curve was an additional aid in identification. A blank curve was also run on the paper. In addition to aiding in identification the curves also enabled a more accurate estimation of the blank. The quantity of the purine was calculated with the values for molar absorbance in 0.1N hydrochloric acid (9, 10, 11). A flow diagram is presented in Figure 1.

Results:

Using the method of Wall (8) separations were made of Manteca molasses and Manteca diffusion juice. Because of the colored materials eluted near the beginning of the fractionation, uracil and other substances acting like uracil could not be detected. With this method, adenine appeared to be the principal constituent in molasses with a considerably lesser quantity of guanine and hypoxanthine. Xanthine was not detected. Xanthine and hypoxanthine were found to be present in the diffusion juice but guanine and adenine were absent, or nearly so. This is different from the work reported by Janacek (3) and Vavruch (4). The former reported guanine as the principal constituent and the latter reported adenine as the principal purine with a lesser quantity of guanine. The amino acid tryptophan

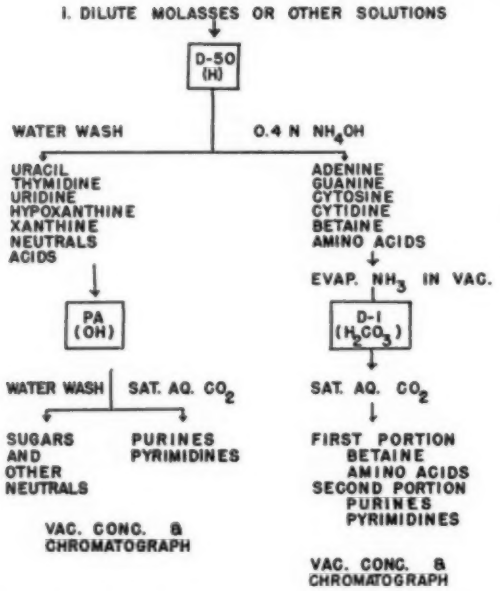

Figure 1.—Ion exchange separation of purine-type compounds.

previously unreported was also found in molasses by the method of Wall (8). Tryptophan was eluted after adenine by the use of 8N hydrochloric acid.

Manteca molasses was treated by the ion-exchange procedure previously described. The fraction not held by the cation resin was treated with the anion resin (Permutit A) and the product chromatographed.

The following compounds were identified from this fraction by the crystallographic properties of their derivatives: uracil, uridine, and hypoxanthine. Thymidine was identified by its chromatographic behavior and ultraviolet absorbance curves in alkaline, acid, and neutral solution. Guanine and adenine were found in the portion eluted from the cation resin and identified by the crystallographic properties of their derivatives.

Table 1.—Purines, Pyrimidines, and Nucleosides in Beet Molasses.

Compound	mg./100 grams non-sugars	mg./l. molasses
Thymidine	15	45
Uridine	210	630
Uracil	7	21
Adenine	150	450
Guanine	100	300
Hypoxanthine	10	30
Unknowns (est.)	15	45

Quantitative data for molasses are given in Table 1. The results are in milligrams per 100 grams of non-sugars and are estimated for a liter of molasses.

Press juice was obtained from a lot of well-mixed cossettes that had been frozen and then thawed with a small amount of water before pressing. The juice was filtered and then treated by the ion-exchange procedure described earlier in this paper. In addition to the compounds found in molasses cytosine and cytidine were identified by their chromatographic behavior and ultraviolet absorption curves. The quantitative data are given in Table 2 and compared with earlier reports on European beets.

Table 2.—Purines, Pyrimidines, and Nucleosides in Sugar Beet Juice (mg./l.)

Compound	Janeck (3)	Vavruch (4)	WURB
Adenine	28	1	40
Guanine	80	3	25
Hypoxanthine	52	4	35
Xanthine	50	5	?
7—Methylxanthine	28	6	?
Uracil		probably	60
Cytosine			5
Guanosine		2	?
Uridine			135
Thymidine			20
Cytidine			10

Several of the compounds in molasses have a high retail value. The retail price of adenine is $2.20 per gram, uridine $4.50 per gram, and thymidine $29.00 per gram. On this basis a liter of molasses has a retail by-product value of $5.00. Probably the compounds could be recovered economically at a price very much lower than this. If a sufficient market could be developed the worth of molasses would be greatly increased.

Summary.

1. Some of the purines, pyrimidines, and nucleosides present in beet molasses have been identified and the quantities determined.

2. A number of the purines, pyrimidines, and nucleosides present in beet juice have been identified and the quantities determined and compared with work on European beets.

3. An ion-exchange method has been developed for separating these compounds from the bulk of the impurities.

4. Uridine, previously unreported, has been found to be the principal constituent of this class of compounds in beet juice and in molasses.

5. Thymidine, cytosine, and cytidine have been found in beet juice.

6. The presence of uracil, reported by Vavruch (4) has been confirmed.

7. Molasses is an attractive commercial source of uridine, adenine, and tymidine.

Acknowledgment: The authors are grateful to Dr. F. T. Jones for the crystallographic identification of the various purines, pyrimidines, and nucleosides.

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Changes in the Concentration of Amino Acids in the Leaves of Sugar Beet Plants Affected with Curly Top

J. M. FIFE[1]

Introduction

Previous investigations (1)[2] have shown that striking differences occur in the relative concentration of certain amino acids in the juice expressed from the leaves of healthy and curly-top-diseased sugar beets. The work reported here summarizes some of the findings obtained at this laboratory on the amino acids found (and the relative amounts of certain ones) in the leaves of healthy, susceptible sugar beets. These are compared to the findings in comparable leaves of plants affected with a severe strain of the curly-top virus.

Preparation of Samples

A variety of sugar beets, susceptible to curly top, was grown in the greenhouse in six-inch pots in highly fertile soil. The plants, four per pot, were inoculated with a virulent strain of curly-top virus as early as the two-leaf and as late as the six-leaf stage, depending upon the experiment, by means of viruliferous beet leafhoppers. The uninoculated plants, thinned to two per pot, were cared for in the same manner and made up the controls. The plants were fertilized immediately after inoculation and at frequent intervals until the leaf samples were taken.

The samples were taken after the disease had reached a maximum degree of severity. This was usually four to five weeks after inoculation, depending upon the age of the plants at the time of inoculation. The oldest leaf showing severe symptoms of curly top, together with all of the younger leaves on the plant, was taken for a sample. Leaves of the same age range were taken from the healthy control plants at the same time. Leaves were taken from a minimum of 20 to as many as 100 diseased plants for each sample of diseased leaves and from smaller numbers of healthy plants for the control sample.

The petioles were removed at the base of the leaf and the juice expressed from the washed, quick-frozen blades to 6000 p.s.i. The juice was filtered through a mat of celite and preserved with thymol and phenyl mercuric nitrate and stored at 10° F. The samples were filtered again immediately before using. Eleven such pairs of samples were prepared over a period of two years.

Analysis by Paper Chromatography

The number of amino acids and their relative amounts were determined by 2-dimensional paper chromatography by the ascending method. Normal butanol-acetic acid-water (4:1:5), phenol-water (4:1) and 2,6-lutidine (200

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[2] Numbers in parentheses refer to literature cited.

ml.), 95 percent ethanol (200 ml.), water (100 ml.) and diethyl amine (6.25 ml.) were the solvents used. As much as 10 /μl of the cleared juice was spotted on 11 x 11 Whatman Nos. 1, 3, and 52 papers for identification purposes.

For quantitative studies, 1-dimensional papergrams were used for all amino acids except histidine, lysine, γ-aminobutyric and glycine. These amino acids, except the last, were determined by 2-directional papergrams using phenol followed by the lutidine solvent. Gamma-aminobutyric acid was separated by dusting the line of ascent of the acids in phenol with basic copper carbonate followed by the lutidine solvent which did not contain the diethyl amine. Glycine was determined using the butanol solvent followed by lutidine. The butanol solvent alone was used to determine valine, the "leucines" and cystine while the lutidine solvent was used to determine threonine and as a check upon the "leucines" and valine. The phenol solvent was used to determine aspartic acid, glutamic acid, alanine, and argenine. The concentration of argenine was so high, relative to that of histidine and lysine, that only 1 /μl of juice was sufficient for a determination of the former and without interference from the other two acids.

Duplicate spots of 1 to 3 /μl of the juice, depending upon the amino acid being determined, of each pair of samples of healthy and diseased leaves were placed side by side 2 cm. apart on all three papers.

The same volume of the standards, shown in Table 1, was replicated four times with each concentration on separate sheets of each paper and run concomitantly in the same cabinet with each set of unidirectional papers.

The papers were developed at a temperature of 23° C., allowing the solvents to ascend, in most cases, 18 to 20 cm. above the origin. The developed papergrams were dried overnight in a circulating oven at 40° C., then dipped into a 0.3-percent solution of ninhydrin in 95 percent ethanol. Color development took place during a six-hour period in an incubator maintained at 40° C., and a relative humidity of 45 percent by passing preconditioned air through the incubator at a rate sufficient to replace the air at least once every two hours.

Table 1.—Composition of Amino Acid Solutions Used as Standards.

Amino Acid	1	2	3	4
			Mg. %	
Aspartic	140	70	35	17.5
Glutamic	140	70	35	17.5
Argenine	140	70	35	17.5
Histidine	200	100	50	25
Lysine	200	100	50	25
Valine	200	100	50	25
Leucine	200	100	50	25
Serine	200	100	50	25
Threonine	200	100	50	25
Gamma-Aminobutyric	200	100	50	25
Glycine	100	50	25	12.5
Cystine	200	100	50	25

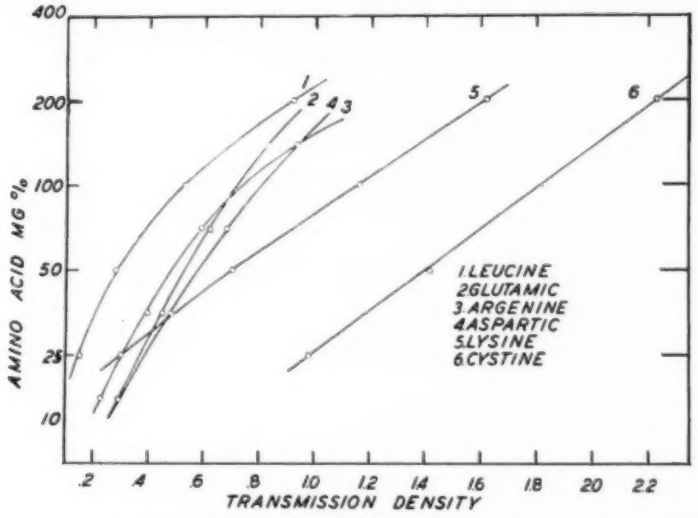

Figure 1.—Typical standard curves obtained by plotting log of amino acid concentration against transmission density.

The relative density of the spots was determined the following morning by a photovolt transmission densitometer unit using a 4-mm. aperture. The transmission density values were corrected for the background value of the paper. The log concentration of the standard amino acids was plotted against the mean "transmission density" of the four spots. Typical curves are shown in Figure 1. The curves were usually straight lines for the three lowest concentrations, but the curves of some of the amino acids tended to flatten somewhat above the 100 mg. percent concentration. The concentration of each amino acid was determined on all three papers (Whatman Nos. 1, 3, and 52) and the papergrams compared as to quality for quantitative purposes. The paper which gave the best separation and sharpest spots was selected for each amino acid for the final determinations. Duplicate spots of each sample on the same paper and duplicate papers were run for the data shown in Table 2.

Table 2 shows the mean concentration of the principal amino acids in the juice expressed from healthy and curly-top-diseased sugar beet leaves in 11 separate experiments, together with the mean ratio of diseased to healthy for each amino acid. The concentration of each amino acid varied somewhat in the 11 samples of healthy juice. This variation could be due to one or more of several factors, such as the difference in the fertility level maintained in the different sets of plants, the age of the plants at the time of inoculation, the variation in the time interval between inoculation, and the taking of the samples, or the time of the year the plants were grown.

Table 2.—Concentration of Certain Amino Acids in the Expressed Juice of Healthy and Diseased Sugar Beet Leaves.

Amino acid	Leaf Juice		
	Healthy Mg. %	Diseased Mg. %	Ratio D/H
Aspartic	35[1]	54[1]	1.55
	41[2]	28[2]	0.67
Glutamic	61[1]	93[1]	1.53
	108[2]	53[2]	0.49
Argenine	50	165	3.3
Lysine	8	26	3.0
Histidine	9	14	1.6
"Leucines"	18	30	1.6
Valine	35	72	2.0
Serine	44	70	1.6
Gamma-Aminobutyric	18	29	1.5
Threonine	23	34	1.4
Glycine	12	44	3.6
Alanine	37	70	1.9
Cystine	10	10	1.0

[1] Mean value of samples taken at 10:00 a.m

[2] Mean value of samples taken at 4:00 p.m.

With the exception of cystine, all the amino acids shown in the table were present in greater concentration in the diseased than in the healthy sample, in 10 of the 11 pairs of samples analyzed. Why the one pair of samples did not follow this same pattern in all respects is not known.

The concentration of aspartic and glutamic acids was found to be influenced by the time of day the samples were taken. These amino acids were higher in the diseased leaves than in the healthy leaves, only in the six samples taken in the morning. The mean ratio of diseased to healthy was 1.55 and 1.53 for aspartic and glutamic acids, respectively. The five samples taken at 4:00 p.m. show that, during the day, aspartic and glutamic acids decreased sharply in diseased leaves while at the same time they increased in the healthy leaves to a degree which completely reversed the ratios found in the samples taken in the morning. The ratio of diseased to healthy for the samples taken at 4:00 p.m. was 0.67 and 0.49 for aspartic and glutamic acids, respectively. From this, it appears that the ability of the beet leaf to utilize these acids during the day is not greatly affected by the disease.

The most striking difference observed is the accumulation of the basic amino acids, argenine, histidine, and lysine in the leaves of the diseased plants. Argenine makes up by far the major portion of this group. The lowest ratio of argenine found in the diseased leaves to that in the healthy was 1.6, while ratios of 4.0, 5.9, 8.1, and in one case, 13.6 were found. In the last instance, however, the argenine in the healthy juice was only 10 mg. percent, which was the lowest value obtained. Glycine accumulated in diseased leaves until the concentration was 3.6 times greater than that found in the healthy leaves. The concentration of glycine, however, was relatively low in the healthy leaves in comparison with that of argenine.

In addition to the amino acids listed in the table, tyrosine, phenylalanine, and methionine have been found in the juice expressed from healthy and diseased sugar beet leaves. At least three other amino acids were present in small amounts. These have not been identified. At least one of the unidentified acids is either a beta or a gamma-amino acid.

Summary

Certain amino acids tend to accumulate in the leaves of susceptible sugar beet plants affected with a severe strain of the curly-top virus. Argenine appears to accumulate to the greatest extent in the diseased leaves relative to that in comparable healthy leaves. A mean of all the determinations showed that the concentration of argenine was approximately three-fold greater in the leaves of diseased beet plants than in healthy leaves. Aspartic and glutamic acids were present in greater concentration in diseased leaves in the samples taken in the morning, whereas the reverse was true for the samples taken late in the afternoon. The average ratio for all the amino acids in the diseased leaves to that in the healthy leaves was greater than 2.0. This indicates that, in general, the amino acids as a whole, tend to accumulate in the leaves of susceptible sugar beets when inoculated with a severe strain of the curly-top virus, to about double that found in comparable healthy leaves. At least three unidentified amino acids are present in the leaves of healthy and diseased beet leaves, one of which appears to be a beta or a gamma-amino acid.

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Zinc Fertilizer Tests on Sugar Beets in Washington[1]

LOUIS C. BOAWN AND FRANK G. VIETS, JR.[2]

Introduction

The recognition by Viets (1)[3] in 1949 that zinc deficiency of both beans and corn was widespread on the irrigated lands of the Yakima Valley and Columbia Basin pointed to the possibility that other field crops would respond to zinc fertilization. Sugar beets are among the other crops on which enough experimental evidence has been obtained to justify some fairly definite conclusions as to their possible benefit from additional zinc. This paper will present the results of a number of observations and experiments made during the past three or four years to determine the response of beets to zinc fertilizers.

Experimental Results

Experiment at Prosser.

In 1952, sugar beets were included in an experiment in which 26 field crops were grown with and without zinc fertilization (2). This experiment was conducted on the Roza Unit of the Irrigation Experiment Station at Prosser at two locations: on an area which had grown zinc-deficient corn the previous year and an adjacent area where growth of corn had been normal. The soil was predominately a Burke very fine sandy loam. In this experiment, the zinc was sideplaced in the same band with nitrogen at the time of planting. Zinc was applied as $ZnSO_4$ at a rate of 23.2 pounds of zinc per acre and nitrogen at 100 pounds per acre as NH_4NO_3. The plot in this experiment was two 23-foot rows of beets, 30 inches apart. One row in each plot was sidedressed with zinc, the other was not. There were four replications for each area.

Although several crops showed deficiency symptoms and responded to both soil-applied zinc and to zinc sprays, sugar beets did not show any indication of poor growth on either the "deficient" or "normal" areas. There was no indication of a response where the beets were sidedressed or sprayed with zinc.

The analysis of leaf blades (Table 1) taken June 24 shows essentially no increase in zinc uptake from the applied zinc. Because of plant-competition variables imposed by different crops bordering treated and untreated rows, no attempt was made to determine the effect of zinc on root yield, but there was no indication that it was affected.

Alkali Soil Reclamation Experiment at Toppenish.

Zinc-fertilizer plots were included in an alkali soil-reclamation project carried out on the Hadley farm near Toppenish, Washington, in 1953. The soil is Umapine fine sandy loam. In an experiment which included plowing to a depth of three feet and gypsum treatments, one-half of each plot

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[1] Contribution from the Soil and Water Conservation Research Branch, Agricultural Research Service, U. S. Department of Agriculture in cooperation with the Irrigation Experiment Station, State College of Washington, Prosser, Scientific Paper No. 1457, Washington Agricultural Experiment Stations, Pullman.

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[3] Numbers in parentheses refer to literature cited.

Table 1.—Zinc Content of Beet Leaves from Beets Grown at Prosser, Washington with and without Zinc Fertilization.

Treatment	Zinc content, p.p.m.	
	"Deficient Area"	"Normal Area"
No Zn	21.5	19.2
Zn applied[1]	21.5	22.8

[1] Zinc was sidedressed at time of planting at a rate of 23.2 pounds zinc per acre in the same band with N at a rate of 100 pounds per acre.

was given an application of $ZnSO_4$ broadcast at a rate of 16 pounds of zinc per acre. The application was made prior to seedbed preparation in the spring.

On August 21, a very definite growth response to the zinc fertilizer could be observed. Where no zinc was applied, the plants were smaller. Leaf blades were reduced in size and yellowish with leaf margins and tips particularly yellow. These symptoms were not present where zinc was applied.

Data based on 20 mature leaf blades sampled at random on August 21 (Table 2) show that zinc fertilizer increased both the weight and zinc concentration of leaves. The analysis of root yields from these plots (Table 2) shows that zinc increased yields about five tons per acre[4].

Zinc Tests by the Utah-Idaho Sugar Company.

In 1954, fertilizer trials were conducted by the Utah-Idaho Sugar Company at eight locations in the Yakima Valley and Columbia Basin which included zinc treatments replicated three times at each location. The zinc

Table 2.—Zinc Concentration in Leaves and Yields of Sugar Beets Grown at the Hadley Farm, Toppenish, Washington.

Treatment[1]	Yield	Dry weight of 20 leaves gms.	Zn in leaves p.p.m.
	tons/acre		
Check	20.4	38.99	7.6
Check + Zn	25.7	47.60	13.0
Gypsum	20.3	32.90	9.6
Gypsum + Zn	23.7	46.15	12.7
Plowed	15.7	—	—
Plowed + Zn	23.9	—	—
Plowed + Gysum	20.3	—	—
Plowed + Gypsum + Zn	22.6	—	—
L.S.D. (.05)	4.0	11.9	3.0

[1] Zinc was broadcast and disked in. The rate was 16 pounds Zn/acre as $ZnSO_4$.

[4] The yield data were obtained from Dr. C. D. Moodie, Associate Professor of Soils at the State College of Washington, Pullman.

[5] Stripping acid residue is a smelter by-product. It is marketed locally under the trade name Zn-M-N-S by the Balfour, Guthrie & Co., Ltd., Seattle, Washington.

was applied broadcast at time of seedbed preparation as stripping acid residue[2] at a rate of five pounds of zinc per acre.

On August 8, leaf-blade samples consisting of 20 leaves were taken from check plots and zinc-treated plots at three locations in the vicinity of Toppenish, Washington.

The analysis of these samples, along with the yield of beets, is given in Table 3. Although significant increases in the concentration of zinc in the leaves occurred, zinc application apparently did not influence beet yields.

Table 3.—The Effect of Zinc Fertilizer on the Yield and Leaf Zinc Content of Beets Grown at Three Locations in the Yakima Valley.

Location	Soil Series	Treatment	Yield[1]	Zn in leaves
			tons/acre	p.p.m.
Andreas Ranch	Wenas	No Zn	23.3	25.7
		Zn Applied	23.6	36.2
Brown Ranch	Ahtanum	No Zn	31.7	24.6
		Zn Applied	32.7	29.5
Moore Ranch	Toppenish	No Zn	28.9	14.9
		Zn Applied	30.9	17.6
Location Means:		Andreas	23.4	30.9
		Brown	32.2	27.0
		Moore	29.9	16.2
Zn Treatment Means:		No Zn	28.0	21.7
		Zn Applied	29.1	27.8
L.S.D. (.05)			5.1	6.9

[1] Credit is due Mr. Don Kidman of the Utah-Idaho Sugar Company for furnishing the yield data and permitting samples to be taken for zinc analysis.

Nitrogen Carrier Experiment at Prosser.

A long-time experiment at Prosser, Washington, on Ritzville fine sandy loam has been designed to study the effects of three nitrogen carriers on plant utilization of both native and applied zinc. Sugar beets were included in this experiment in 1955. The treatments in this experiment have been five levels of zinc—0, 2, 4, 8, and 16 pounds per acre, in factorial combination with three nitrogen carriers, each at three rates. The zinc treatments were made only once, at the start of the experiment in 1953, and were broadcast. The nitrogen treatments are applied annually.

Data are given in Table 4 from certain treatments selected to show the effect of different levels of zinc on beet yields and zinc content of leaf blades. Leaf samples were taken for analysis on August 20, the sample consisting of 20, young, fully-developed leaves without petioles. The results show that, although the high levels of zinc application raised the leaf zinc to 36.2 p.p.m., as compared with 21.7 p.p.m. where no zinc was applied, it did not affect the yield of beets.

Table 4.—Effect of Zinc on Yield and Leaf Zinc Content of Sugar Beets Grown at Prosser, Washington, 1955.

Treatment[1]		Yield	Zn in leaves
N Carrier	Zn rate		
	Pounds Zn/acre	tons/acre	p.p.m.
NH_4NO_3	0	30.61	20.2
	2	28.34	29.5
	4	30.41	26.1
	8	29.89	28.1
	16	29.64	34.8
$Ca(NO_3)_2$	0	31.55	23.2
	2	27.36	27.7
	4	29.59	27.5
	8	32.33	27.6
	16	30.00	37.6
L.S.D. (.05)		N.S.	7.9

[1] The Zn treatments were made in 1953 as a broadcast application of $ZnSO_4 \cdot 7H_2O$. In 1953 the plots were cropped to grain sorghum, in 1954 to potatoes. For both N carriers the rate was 160 pounds N applied every year.

Some Miscellaneous Observations.

Various trials which have been made since 1950 indicate that, even in situations where growth of beets is poor, zinc is not the limiting factor. Several attempts to improve the beet growth on "cuts" by spraying the plants with a $ZnSO_4$ solution have been unsuccessful. In 1951, on a Roza farm north of Prosser, poor growth of sugar beets was observed adjacent to a field of Red Mexican beans showing typical zinc-deficiency symptoms. Zinc sulfate sprays were applied to replicated plots of both the beans and the sugar beets. The beans showed an excellent vegetative response, whereas the growth of beets was not affected. No yields were taken.

In 1954, stunted beets, growing on a cut area of Warden-Wheeler soil near Wheeler, Washington, in the Columbia Basin, were sidedressed with $ZnSO_4$ and NH_4NO_3 as a diagnostic procedure. The material was applied about three inches deep and about three inches from the center of the beet row shortly after thinning. A continued poor growth of these beets and low yield indicated that other factors were the primary cause of the stunting.

In 1955, two "lime-knobs" just north of Prosser, Washington, on Sagemoor soil, where poor growth of crops had been observed for several years, likewise showed no visible improvement in the growth of sugar beets when a strip across each knoll was given a heavy application of zinc (20 pounds per acre) prior to planting.

Discussion

The experimental evidence obtained to date would seem to justify several fairly definite conclusions with respect to the use of zinc fertilizers on sugar beets in the state of Washington. First, it has been shown that, for the most part, soils of the Yakima Valley and Columbia Basin can be expected to supply sufficient zinc for high yields of beets. The one exception, perhaps, is where beets are grown on high pH soils such as the saline-alkali

area in the vicinity of Toppenish. On the Hadley farm, where the results of Table 2 were obtained, the surface soil pH is likely to be 9.5 or higher.

The second conclusion which these data support is that the zinc concentration of young, fully-developed leaf blades taken in mid-August must be below approximately 10 p.p.m. before the beet plant will show zinc deficiency symptoms or respond to zinc fertilization. Most of the leaf samples analyzed show a zinc content well above this figure. An interesting sidelight is that at the Moore Ranch (Table 3), where beet leaves from check plots had a zinc concentration of 14.9 p.p.m., corn grown in 1955 showed a spectacular vegetative response to the zinc applied the previous year.

Finally, there is evidence that sidedressing is not a satisfactory means of application for getting zinc uptake by sugar beets. Zinc applied in this manner, because of its immobility in the soil, is probably above the main rooting area of the sugar beet most of the growing season. Plowing down the zinc seems to offer the best assurance that zinc fertilizers will be contacted by the root system.

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Results of Use of the Red-Marker Beet as a Top Cross Parent

D. D. Dickenson and D. F. Peterson[1]

In order to obtain inbreds of high combining ability, some method must be used for efficient production of identifiable top crosses, the latter being a measure of general combining ability (2, 3)[2].

Male-sterile populations present no particular problems, as far as testing for their combining ability, since the number of usable male-sterile lines is limited by the intricacies of their isolation and the cross is identifiable as the seed produced. Consequently, several top crosses of male steriles with the top-cross tester parent can be made in one seed isolation.

With pollen-producing inbreds, however, the number should not be limited in order to increase the probabilities of isolating the highest combining inbred. Testing these pollen producers for combining ability, however, presents the problem of producing a large number of top crosses with identifiable crosses.

For pseudo self-fertile beets, a top-cross parent with a dominant marker character has been used to produce crosses which could be identified in the seedling stage (1, 4).

This paper reports a study of the use of the half-sugar, half-red garden beet, referred to as the red-marker beet, as a top-cross parent.

Material and Methods

Nine pseudo self-fertile varieties of sugar beets were selected from previous tests for high sucrose, high tonnage, leaf-spot resistance and curly-top resistance. It was desired to further inbreed within the most desirable of these varieties for high-sucrose, high-combining inbreds. The red-marker beet was used as a top-cross parent with each of the varieties and selection of the sources, in which to continue further work, was to be based on the top-cross performance.

Roots of each variety were planted so that every root had a red-marker beet on all four sides. Seed was harvested from approximately 50 plants of each variety and bulked according to varieties.

The amount of crossing was determined from germinations and the seeding rate adjusted accordingly. At thinning time, the top crosses were thinned to the intermediate red plant color of the cross, except where there were no crosses within the desired thinned spacing. In such cases a green plant was left. The degree of crossing differed among varieties, depending on coincidence of flowering times and on the degree of self sterility of plants within the varieties.

The field tests were conducted at Sheridan, Wyoming, Sidney, Montana, and Tracy, California, in 1955. Design of the tests was rectangular lattice at Sheridan and randomized block at Sidney and Tracy. Yield determinations were made from the entire plot of one row, 50 feet long. The tests at

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[1] Plant Breeder and Pathologist, Holly Sugar Corporation, Tracy, Calif., and Plant Breeder, Holly Sugar Corporation, Sheridan, Wyoming, respectively.

[2] Numbers in parentheses refer to literature cited.

Sheridan and Sidney were on 22-inch rows and the one at Tracy was on 30-inch rows. Two samples of 15 beets each were taken per plot for sucrose determination at Sheridan and Sidney and two 12-beet samples per plot were taken at Tracy.

At harvest time, all green beets, which had been left at thinning time for growth competition, were removed from the top-cross plots and corrections were made for missing portions of plots.

Data presented have been extracted from yield tests with 30 entries.

Experimental Results

Table 1 presents the tonnage yields of the varieties and their top crosses with the red-marker beet. Differences will be noted among the varieties, among the top crosses, and between the individual varieties and their top crosses.

At Sheridan the top crosses of the red-marker beet with US 75, 00263-0, 20648-0, and 30208-0 exceeded the yield of the respective varieties alone, while MW 391 exceeded its top cross.

At Sidney the crosses of H 18-40, 00263-0 and 20648-0 exceeded the yield of their respective varieties.

At Tracy, a high incidence of curly-top infection markedly influenced the tonnage yields. This occurrence probably accounts for the high yields of US 22/3 and US 75, while their crosses are considerably lower. The yield of the MW 391 top cross was also lower than MW 391, although this reduction could not be explained by a difference in percent of curly-top infection. The yield of the 30208-0 top cross exceeded the yield of that variety alone.

Table 1.—Tonnage Yield of Varieties and the Crosses with the Red-Marker Beet at Three Locations, 1955.

	Yield in Tons Per Acre							
	Sheridan, Wyoming		Sidney, Montana		Tracy California		Tracy, Percent Curly Top	
Variety	Variety	Top Cross	Variety	Top Cross	Variety	Top Cross	Variety	Top Cross
Red Beet	11.381		14.481		14.971		94.8	
US 22/3	19.253	19.316	20.232	20.744	40.621	26.611	3.1	78.0
US 75	18.129	20.745	20.714	21.772	39.918	32.982	1.7	69.4
Klein "E"	21.885	22.870	24.432	25.223	25.762	23.760	92.9	92.6
MW 391	22.654	19.743	23.556	22.310	25.725	20.601	90.6	89.6
H 18-40	19.042	18.560	19.228	22.403	23.612	19.926	92.7	89.8
00263-0	16.560	22.469	19.578	24.424	23.366	20.977	96.7	93.6
7017-0	18.574	18.320	18.661	20.555	21.067	17.880	91.4	91.2
20648-0	17.703	19.760	17.609	22.152	19.963	22.304	92.1	90.2
30208-0	16.312	22.008			14.744	22.577	92.1	94.2
3124-0			21.840	22.245				
LSD .05	1.889		2.115		3.881			
r (n=9)	—.12[1]		+.48[1]		+.77[2]			
r (n=7)					—.90[3]			

[1] Denotes non significance.
[2] Denotes significance at 5 percent level.
[3] Denotes significance at 1 percent level.

The correlation coefficients calculated for the yield of the varieties with their top crosses to the red-marker beet are also indicated in Table 1. At Sheridan ($r = -.12$) and Sidney ($r = +.48$), r values were nonsignificant. At Tracy ($r = +.77$), the relationship was significant at the 5 percent level. Omitting US 22/3 and US 75 and their crosses because of differential curly-top infection, the correlation coefficient ($r = -.90$) is significant at the one percent level.

Table 2.—Percent Sucrose Content of Varieties and Top Crosses with the Red-Marker Beet at Three Locations, 1955.

	Sheridan, Wyoming			Sidney, Montana			Tracy, California		
	Percent Sucrose		Difference from	Percent Sucrose		Difference from	Percent Sucrose		Difference from
Variety	Variety	Top Cross	Mean of Parents	Variety	Top Cross	Mean of Parents	Variety	Top Cross	Mean of Parents
Red Beet	10.21			8.84			11.48		
US 22/3	15.41	13.07	+.26	15.89	11.82	—.55	13.40	10.83	—1.61
US 75	15.34	12.84	+.06	15.41	12.38	—.25	12.92	10.33	—1.87
Klein "E"	15.60	12.51	—.40	16.04	12.42	—.02	13.79	11.17	—1.47
MW 391	15.56	12.70	—.19	16.07	12.74	+.28	13.53	10.51	—2.60
H 18-40	16.28	13.19	—.06	16.78	12.78	—.03	13.61	11.98	—.57
00263-0	15.54	12.57	—.31	16.12	12.63	+.15	14.05	11.66	—1.11
7017-0	15.77	12.85	—.14	15.82	12.08	—.25	13.50	11.46	—1.03
20648-0	16.88	13.51	—.04	17.86	13.22	—.13	15.06	12.17	—1.10
30208-0	17.78	13.75	—.25				15.36	12.29	—1.13
3124-0				16.34	12.54	—.04			
LSD .05		.51			.69			1.24	
r (n=9)		+.89[3]			+.78[2]			+.82[2]	
r (n=7)								+.72[1]	

[1] Denotes non significance.
[2] Denotes significance at 5 percent level.
[3] Denotes significance at 1 percent level.

Table 2 contains the percent sucrose content of the varieties and their top crosses with the red-marker beet. Also presented are the differences, for the crosses, above or below the arithmetic mean of the two parents. At Sheridan and Sidney, the sucrose content for the crosses is very near the arithmetic mean of the parents, with higher sucrose varieties having higher sucrose top crosses. The top crosses at Tracy, however, are markedly below the expected figure of the arithmetic mean of the two parents. The higher sucrose varieties still have the higher sucrose top crosses, however.

Discussion

If the red-marker beet is taken as a suitable top-cross parent for indicating general combining ability, then those varieties which, in top-cross combination, exhibit a yield in excess of the varietal parent should be ones having dominant favorable yield factors not found in the red-marker beet parent.

It remains to be seen if the top-cross yields, which were in excess of the varieties, are a manifestation of general or specific combining ability. The nonsignificant correlation coefficients found at Sheridan and Sidney

would indicate that the yield of a variety *per se* could not be taken to indicate which ones contain the higher proportion of plants that are better general combiners.

The correlation at Tracy (r = +.77) was calculated including US 22/3 and US 75. As indicated in Table 1, there was a difference in curly-top incidence on these varieties and their top crosses. When they were omitted, the correlation changed to a negative value (r= —.90), indicating that the higher yielding varieties actually are made up of plants with low general combining ability. A notable exception to this is 30208-0. The negative relationship expressed by the r value is due to the lower yield of most of the top crosses compared with their parental variety yield. The lower top-cross yields, as compared to variety yields, could be due to a differential response to curly-top infection.

Sucrose content of the top crosses followed a pattern experienced in previous studies by many workers (i.e. the sucrose content of the top crosses was intermediate between the sucrose contents of the two parents). The results at Tracy under heavy curly-top infection were the notable exception to this. Here again, this could possibly be a differential response to comparable amounts of curly-top infection.

Summary and Conclusion

1. The red-marker beet with a dominant red plant color was used as a top-cross parent on nine varieties of pseudo self-fertile sugar beets. The top-cross plants were identifiable in the seedling stage and left at thinning time.

2. Yield data obtained from the varieties and their top crosses revealed that certain varieties at the three locations produced top crosses whose yields exceeded the yield of the varietal parents. This was taken to indicate that the varieties were high in general combining ability. However, future tests will show whether specific or general combining ability was responsible for the results obtained.

3. Correlations (r = —.12, +.48, —.90) between yields of varieties and their top crosses at the three locations indicated, in general, that the yield of the variety *per se* could not be taken as an indication of the level of general combining ability of plants within the variety.

4. Sucrose content, in general, was intermediate between the two parents, with higher sucrose parents yielding the higher sucrose top crosses.

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Determination of Floc in Refined Sucrose by Coagulation with Quarternary Amines

J. R. Johnson and M. R. Diehl[1]

Introduction

The various methods of measuring the amount of floc material present in refined sugar have not been entirely satisfactory. A control method is needed which is rapid and which has a means of quantitatively assigning a value of the amount of floc material present. Older methods for laboratory control were based on visually estimating the amount of floc and required a reaction period of at least twenty-four hours.

There have been some very good methods devised through which the floc material can be extracted quantitatively and identified (1), (2)[2]. These methods require a considerable amount of technique and require the time of the analyst to the point where they are out of the scope of an ordinary control laboratory.

The Quaternary Amine method has been developed. It is not time consuming and can readily be used for control purposes. It is rapid and does not rely upon the operators visual estimation of floc actually present, since the measurement is taken on a spectrophotometer or on a colorimeter capable of measuring transmittance at 720 $m\mu$.

Floc material extracted from refined sugar has been identified as saponins or a glycoside consisting primarily of glucuronic and oleanolic acids or their salts (1). Saponins are largely removed by carbonation but a small amount does at times get past the purification steps and hence into the sugar crystal itself. Saponins present in crystallized sugar are of colloidal size when the sugar is dissolved. These colloids are negatively charged and hence can be coagulated by cationic compounds. Hyamine 10-X No. 1622 or Roccal will bring about coagulation under the proper conditions. This characteristic is the basis of the method developed.

This method is a modification of Dr. Galvin's preliminary quaternary amine titration and Holly's preliminary method of excess Roccal addition. The method is placed on a quantitative basis through use of curves based on sugars with a known amount of saponin added.

Preparation of Saponin for Basic Standardization.

Several procedures for extracting a saponin material from sugar beets were investigated. The method recommended produced a material which is easily reproduced and which, when used in the floc method, is comparable to saponin material supplied by the Western Utilization Research Laboratory for our preliminary work. The product extracted is not a pure compound but a mixture of compounds which is representative of the actual floc material in sugar beets. It also has characteristics which should make it suitable for analysis of cane sugars as well as beet sugar. It is light brown or tan in color and is easily pulverized to make handling easy.

Procedure.

Select one or more sugar beets which are in good condition. Peel the outer skin from the beet. This peeling should be at least one-eighth inch

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[2] Numbers in parenthesis refer to literature cited.

thick but avoid peelings which are over one-fourth inch. Discard the peeled beet. Cut the peelings into small pieces and place them in a Waring Blender. Add water equal to about one-fourth the weight of the peelings and blend for five minutes.

Extract the blended material with water in a Soxhlet Extraction apparatus for three to four hours. Extraction must be carried out in batches until one or two liters of liquid is obtained. The pulp is discarded. The extract contains sugar, glycosides, and other soluble non-sugars which are contained in the skin of the beet. Since the saponin is concentrated in the skin of the beet this procedure is preferable to using diffusion juice as the starting point.

Acidify the liquid to 1.5 pH with HCl and heat to 90°C. for one hour. Allow the acidified liquid to stand overnight. Filter the juice through a Buchner funnel, using a small amount of filter-aid to speed up filtration. Wash the cake with acidified water to remove sugars. Discard the filtrate and washings. Dry the cake with infra-red lamps. Do not heat the cake too much or decomposition of the material may cause trouble.

Extract the dried cake with ethanol or with special denatured ethyl alcohol for six hours. This also must be a batch operation. Concentrate the extract to a small volume (50 ml. or less) and pour into a larger volume of acidified water (3000 ml.). Let this solution stand overnight. A white flocculent precipitate will form.

Filter the solution through a white filter paper which is tight enough to retain the precipitate but which has good flow rates. Do not use filter-aid.

Dry the precipitate carefully and dissolve it with hot alcohol by pouring the alcohol through the paper. Use only enough alcohol to dissolve the precipitate and wash the paper slightly. Evaporate the alcohol solution slowly in an evaporating dish. The brown material remaining can be readily scraped from the dish after it is dried. This material is pulverized and bottled for use. This is the saponin material for standardization.

From one beet of average size about 500 to 1000 mg. of material can be obtained.

Standardization of Floc Method.

(1) Accurately weigh out 0.0250 grams of the saponin material. Dissolve the saponin in alcohol (heat slightly if necessary) and make up to a total volume of 250 ml. with alcohol (Bakers Reagent Alcohol, Denatured No. 3A). 1.0 ml. of this solution contains 0.1 mg. of saponin.

(2) Dissolve 2.0 grams of Hyamine 1622 (10-X) in distilled water and dilute to one liter (0.2 percent solution).

To each of the eighth 100 ml. Kohlrausch flasks add 10.0 grams of sugar which is known to be floc free. Dissolve the sugar in a small amount of water and add reagents according to the following schedule.

Flask Number	ml. Saponin Solution	ml. 10-X	p.p.m. Saponin Present
1	0	0	0
2	0	10	0
3	1.0	10	10
4	2.0	10	20
5	4.0	10	40
6	6.0	10	60
7	8.0	10	80
8	10.0	10	100

Complete all volumes to 100 ml. with distilled water. Mix solutions in each flask by inverting them 8 to 10 times. Be careful not to use a vigorous action as air bubbles will be formed.

Place flasks No. 1 through No. 8 inclusive in an 80°C. water bath for one hour.

Cool the flasks to 20°C. and adjust volumes to 100 ml. to make up for evaporation. Mix the contents of the flasks by inverting them 10 times. The mixing procedure must be carefully done and it must be exact as the coagulation of the saponin does not come to an equilibrium unless adequate mixing has been achieved. The same technique must be followed when determining floc in an unknown sugar.

The solution in flask No. 1 is the reference solution. Read the percent transmittance at 720 $m\mu$ for all flasks. Check each reading carefully and make an average of at least three readings. The entire procedure of standardization should be repeated two or three times and the average taken for a more accurate standardization.

Results are then plotted on regular graph paper. (Figure 1). The graph should be a straight line if all of the work was carefully done. From the graph a table may be prepared for use in the subsequent analysis.

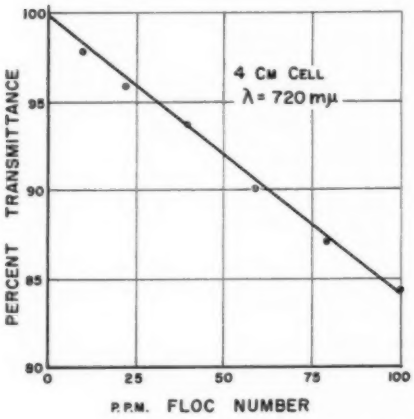

Figure 1.

Analytical Procedure.

After having previously constructed a standardizing graph or table as above the actual analysis is simple.

(A) Weigh 10.0 grams of the sugar under test into each of two 100 ml. sugar flasks. Dissolve the sugar in about 50 ml. distilled water.

(B.) To the first flask add distilled water up to the 100 ml. mark, mix the solution by inverting the flask 10 times carefully.

(C.) To the second flask add 10 ml. of the 4.2 percent Hyamine 1622 solution. Fill to the mark with distilled water. Mix contents by inverting 8 times and place the flask in a water bath at 80°C. for one hour. Cool the flask and re-adjust the volume. Mix the contents by inverting 10 times.

(D.) Find the percent transmittance of the solution at 720 m_μ against the reference solution. From the table or graph find the floc number of the sugar.

Remarks.

In developing the procedure we stated previously that several methods of extracting the saponin material were employed. We used both ether and alcohol as extracting solutions and obtained materials from both extractions on the same acid precipitated material, alcohol followed by ether and vice-versa.

All substances recovered were found to act in a similar manner when their alcoholic solutions were used as standardizing reagents. A statistical analysis did point out that the transmittance values found were significantly different when all of the various extracted substances were compared.

We found, however, that our straight alcoholic extraction was comparable to saponin material furnished by Western Utilization Laboratory. The differences were not significant at 90 percent confidence levels.

We also found that the straight alcoholic extracted material could be duplicated. This led to the procedure adopted for the floc method presented.

Since the standardizing material produced in this manner compares with saponin produced under different conditions in a different laboratory when checked by our proposed method we feel that we can recommend the method for routine laboratory control of floc in sugar.

Average values (transmittance) found by using a few of the various materials are shown in Table 1. Statistically there is no significant difference between Alcohol Ex. 55-1, Alcohol Ex. 55-2 and WR-54.

Table 1.—Transmittance Values for Various Extracted Materials.

(4 Cm. Cell Depth)

p.p.m. Saponin	Alcohol Ex. 55-1	Alcohol Ex. 55-2	WR 54	AX 54	Ether Ex. 55	Ether Alk. Ex. 55
10	97.75	98.33	97.83	98.10	98.13	98.60
20	96.63	96.50	96.33	96.75	96.75	97.90
40	92.88	92.33	92.67	93.88	93.63	95.70
60	89.13	88.50	89.50	90.63	90.63	94.20
80	87.00	84.67	86.83	87.50	87.75	92.00
100	84.50	82.00	84.50	85.38	84.75	90.00

Correlation of the acid-reflux method with the method presented here is difficult. Visual estimation of floc depends upon size and number of particles but does not allow for density. Table 2 illustrates this lack of correlation.

Table 2.—Lack of Correlation Between Two Methods.

Sample	1	2	3	4	5
Visual Grade	2	4	4	1	1
Floc Number (Colorimeter)	12	23	20	27	6

The floc grade of Sample No. 4 was of very fine, evenly diispersed floc. According to instructions it could only be given a grade of 1, however, the colorimetric method shows a fairly heavy concentration of floc.

It is interesting to note that the turbidity developed in the new method does not vary in particle size. It varies only in the number of particles and is stable for long periods of time.

Mixtures of two samples of sugar, known to have a marked difference in floc content, show a promise of accuracy in the colorimetric method (3).

Figure 2 shows the results of this test.

Analysis made on cane sugars also indicate the presence of colloids. Dissolved cane wax behaves in a manner similar to sugar beet saponin.

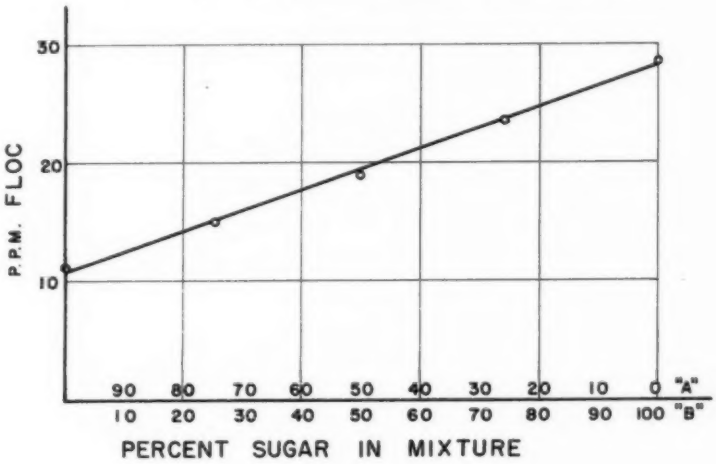

Figure 2.

Conclusions

This method is readily adopted to refinery control. It is rapid and places the floc or saponin content of granulated sugar on a quantitative basis.

Analysis of sugars produced this season show a range of floc from 5 to 27 p.p.m. We have not set a maximum allowable floc value for specific trade sugar as yet. However, sugar produced for bottling purposes has been found to be suitable which has a floc value as high as 12 p.p.m. Generally speaking, floc under 10 p.p.m. will be graded as "O" floc by the older methods.

Acknowledgment

In developing this method many useful suggestions and aid in evaluation were made by Spreckels Sugar Company, Holly Sugar Corporation, and the Western Utilization Research Laboratory.

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Influence of Pre-Emergence Irrigation, Planting Date, and Planting Method on the Production of Sugar Beets in Western Nebraska.[1]

LIONEL HARRIS, R. R. ALLMARAS, H. F. RHOADES, F. V. PUMPHREY[2,3]

Insufficient early spring precipitation sometimes reduces the effective growing season for sugar beets in western Nebraska by delaying germination of sugar beet seed and emergence of the seedlings. Pre-emergence irrigation and moisture conserving planting methods may achieve germination and emergence of seedlings independent of precipitation. These methods might increase the value of early planting, particularly when temperatures are favorable for plant growth.

Nuckols (1)[4] found that early planted sugar beets yielded two to four tons more per acre than those planted at medium or late dates. He concluded that frost damage to early planted beets was less hazardous than insufficient moisture for germination of late planted beets.

Experiments dealing with pre-emergence irrigation, a moisture conserving planting method, and planting date were conducted from 1948 to 1953 at the Scotts Bluff Experiment Station. The results of experiments conducted in 1948 and 1949 were reported previously (2). The results from the six-year study are reported here.

Experimental Procedure

This study was conducted on Tripp very fine sandy loam maintained in a high state of productivity. Alfalfa was plowed under as green manure two to three years previous to the sugar beet crop, and a 15-ton per acre application of manure was made on each experimental area before planting.

The treatments studied were as follows: Three planting dates (early, medium, late); two planting methods (surface and ridge-cover); and two pre-emergence irrigation practices (no irrigation and irrigation after planting). The treatments were arranged in a split plot design with six replications of each treatment during the first four years, and four replications during the last two years. The smallest plot unit was four rows spaced 20 inches apart and 150 feet long (1948-51) and 100 feet long (1952-53).

The mean dates of early, medium and late planting and standard deviations were, respectively, as follows: April 2 ± 4 days, April 16 ± 3, and May 2 ± 1.

Surface planting is the method used commonly by sugar beet growers. The ridge-cover planting method consisted of surface planting with a ridge or mound thrown over the row with cultivator disks. The ridges were

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[1] Contribution from the Nebraska Agricultural Experiment Station and the Western Soil and Water Management Section, Soil and Water Conservation Research Branch, U. S. Department of Agriculture. Published with approval of the Director as Paper No. 745, Journal Series, Nebraska Agricultural Experiment Station. This study was supported in part by the Beet Sugar Development Foundation.

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[3] The authors are indebted to H. Juergens, Manager, The Great Western Sugar Company, Mitchell, Nebraska, for sucrose analyses.

[4] Numbers in parentheses refer to literature cited.

removed with a harrow or cultivator blade following germination of the sugar beet seed. The purpose of the ridge was to conserve moisture near the sugar beet seed. Surface planting was studied six years (1948-1953) and ridge-cover planting four years (1948-1951).

Water for pre-emergence irrigation was applied between every other row of beets in the surface planting method, and between each row in the ridge-cover planting (Figure 1). In each case sufficient water was applied within two days of planting to provide adequate soil moisture for germination of beet seed.

The seed bed for the experimental area was spring plowed and uniformly prepared previous to the early planting date. After the beets were thinned, all plots received the same cultural practices for the remainder of the season.

Figure 1.—Pre-emergence irrigation of surface and ridge-cover planted sugar beets is shown on the immediate left and right, respectively.

Experimental Results and Discussion

Analyses of variance were made on each year's data. Statistically significant effects of single factors based on these analyses were as follows: (A) Planting date influenced yield of roots and harvest stand each year. (B) Pre-emergence irrigation occasionally affected yield of roots and harvest stand. (C) Planting method influenced yield of roots and harvest stand more frequently than irrigation for emergence. (D) Sucrose content was not affected by any single treatment studied. The two-factor interactions of planting date by pre-emergence irrigation and planting method by pre-emergence irrigation were significant more frequently than other interactions. Therefore, pre-emergence irrigation and planting date are discussed under. (a) the surface, and (b) ridge-cover methods of planting.

Six-year treatment means did not differ significantly due to the large mean square for treatment by year interaction. The standard error of the difference between six-year treatment means ("Students'" test of significance

for paired variates (3), was used to show that there are no significant differences between these means. This method eliminates correlation between values in any one year.

Surface Planted Sugar Beets.

Influence of Planting Date: Sugar beets planted May 2 produced approximately two tons less roots per acre than beets planted in early or mid-April (Table 1). This trend prevailed during most of the individual years.

Stands of early planted beets were the poorest, while stands from the late planting were slightly lower than those of beets planted April 16. Soil crusting was a greater problem with late than early or medium planted sugar beets.

Value of Pre-emergence Irrigation: Irrigation for emergence did not influence the yield of sugar beets during the six-year period as a whole. The six-year mean yields were essentially the same at any one planting date whether or not the beets received an early pre-emergence irrigation (Table 1). During individual years, however, the crop benefited or suffered significantly from the early irrigation. For example, in 1948 sugar beets planted early and irrigated for emergence yielded 4.8 tons more per acre than those planted the same day and not irrigated, but in 1949 and 1951 yields were respectively 2.2 and 3.8 tons per acre lower where irrigated for emergence. In 1948, the early planted and early irrigated beets emerged ahead of those not irrigated and did not suffer frost damage, whereas in 1949 and 1951 frost severely damaged sugar beet seedlings in this treatment. The non-irrigated beets escaped frost damage in 1949 and 1951 because of slower germination and emergence.

During some years precipitation occurred shortly after planting and obliterated any effects pre-emergence irrigation might have shown. This was particularly significant at the late planting date where the yields indicate no influence of irrigation for emergence. Each year during the six-year period, adequate precipitation to germinate beet seed occurred within five days after the late planting date.

Pre-emergence irrigation for beets planted in mid-April improved yields slightly in 1948, 1949, 1950, and 1952. This circumstance may be associated with a slight improvement in stands and a slight increase in growing season during these years as a result of irrigation for emergence. The differences, however, were not statistically significant.

Irrigation for emergence at the early planting date was detrimental to stands five years in six. Irrigation hastened emergence about ten days, but this circumstance was not always advantageous. In several instances, particularly 1949 and 1951, this irrigation hastened emergence only to have many of the beet seedlings killed by frost.

The best stands were obtained with beets planted April 16 and irrigated for emergence. The positive effects of irrigation for emergence for all years except 1953 were related to a deficiency of rainfall after the medium planting date. Pre-emergence irrigation was not beneficial to stands in 1953 because of unfavorable temperatures during the emergence period. In 1952, pre-emergence irrigation was the most beneficial due to the absence of rainfall during the emergence period.

Table 1.—Influence of Pre-emergence Irrigation and Planting Date on Yield of Roots, Harvest Stand and Sucrose Concentration in Surface Planted Sugar Beets (1948-1953). Scotts Bluff Experiment Station.

Year	Pre-emergence irrigation practice	Mean Planting Date								
		April 2	April 16	May 2	April 2	April 16	May 2	April 2	April 16	May 2
		Yield of Roots (tons/A)			Harvest Stand (%)[1]			Sucrose Concentration (%)		
1948	Irrigation	24.1	22.1	19.6	88	89	82	14.4	15.2	15.2
	No irrigation	19.3	21.3	19.1	82	86	77	14.9	15.0	15.1
1949	Irrigation	19.5	23.4	22.0	50	90	86	16.8	17.2	17.5
	No irrigation	21.7	22.7	22.5	62	86	89	16.9	17.3	17.4
1950	Irrigation	26.5	24.7	23.1	70	82	77	18.0	17.7	18.4
	No irrigation	25.6	23.3	23.6	62	80	78	18.5	18.1	18.2
1951	Irrigation	14.9	17.5	16.6	45	75	69	16.2	17.0	16.8
	No irrigation	18.7	18.0	16.2	75	71	67	16.8	16.5	16.6
1952	Irrigation	23.9	23.8	19.7	87	100	96	15.7	15.0	15.7
	No irrigation	24.7	22.8	18.5	93	89	92	15.6	15.4	15.6
1953	Irrigation	20.2	20.2	20.4	72	96	93	16.2	17.6	16.6
	No irrigation	20.8	22.4	19.1	82	102	81	16.9	17.3	17.7
Mean (6 years)	Irrigation	21.5	21.9	20.2	69	89	84	16.2	16.6	16.7
	No irrigation	21.8	21.8	19.8	76	86	81	16.6	16.6	16.8
Standard Error of the difference between means ("Students'" paired variates)		1.20	0.56	0.37	6.6	2.7	6.3	0.22	0.14	0.20

[1] 100 percent stand = one beet plant per foot of row in 20-inch rows.

Despite the fact that poorest stands occurred with beets planted early, yields were the same as for beets planted the middle of April, and were two tons per acre higher than yields from the late planting. In 1949 early planted pre-emergence irrigated beets yielded 19.5 tons per acre with a stand of only 50 percent, and in 1950 the yield was 26.5 tons with a stand of 70 percent. Apparently early planting and early emergence compensated to some extent for loss in stand.

Neither planting date nor pre-emergence irrigation influenced the sucrose percentage of sugar beets in any one year or during the six-year period (Table 1). Growing conditions prevailing during different years had a much greater effect on sucrose content than the treatments studied. Favorable or unfavorable conditions for sucrose accumulation affected all treatments approximately the same in any one year. In 1948 the beets contained approximately 15 percent sugar, and in 1950, 18 percent. Apparently 1950 was a favorable year for the production of high yields of beets which contained a high percentage of sugar. High or low sucrose concentration was not significantly associated with good or poor stands in these tests.

Ridge-Cover Planting.

Influence of Planting Date and Pre-emergence Irrigation: Yield trends under the ridge-cover method of planting were similar to those under the surface method of planting where beets were pre-emergence irrigated. Planting date had little influence on the mean yields of ridge-cover planted beets not irrigated for emergence (Table 2). Irrigation for emergence at the early and medium planting dates increased yields slightly over beets not irrigated.

Inferior and generally more erratic stands were associated with ridge-cover planting more than with surface planting. This circumstance was related to difficulties encountered in removing ridges satisfactorily. In some instances rain delayed removal of the ridges at the proper time, and in others, hot weather following removal produced heavy crusts over the beet seedlings. The implements used—spike tooth harrow and cultivator blade—did not perform the operation as desired. Too much or too little soil was removed in some situations. The difficulties encountered in removing ridges influenced stands and the distribution of plants within a particular stand of sugar beet plants.

Sucrose percentage was not influenced by the ridge-cover method of planting or treatments associated with ridge-cover. However, sucrose percentages of beets varied more under the ridge-cover than under the surface method of planting.

Comparison of Surface and Ridge-Cover Methods of Planting Sugar Beets.

Differences in the yields of beets from the two methods of planting were small at each planting date (Table 3). The yields under the ridge-cover planting method were lower in each instance. Stands were lower under the ridge-cover than under the surface method of planting at each planting date. Under the conditions of these experiments, the lower yields and stands reflect the inability to satisfactorily remove the ridges.

Table 2.—Influence of Pre-emergence Irrigation and Planting Date on Yields of Roots, Harvest Stand, and Sucrose Concentration of Ridge-Cover Planted Sugar Beets. 1948-1951, Scotts Bluff Experiment Station.

Year	Pre-emergence irrigation practice	Mean Planting Date								
		April 2	April 16	May 2	April 2	April 16	May 2	April 2	April 16	May 2
		Yield of Roots (tons/A)			Harvest Stand (%)[1]			Sucrose Concentration (%)		
1948	Irrigation	24.6	22.1	18.5	75	82	66	14.6	15.1	14.6
	No irrigation	19.4	21.7	21.9	48	86	86	15.1	14.9	14.5
1949	Irrigation	20.6	23.2	21.6	54	86	84	16.6	17.5	17.2
	No irrigation	20.6	20.8	19.3	82	79	70	17.0	14.7	17.3
1950	Irrigation	24.0	22.9	21.5	58	50	66	17.7	17.8	18.3
	No irrigation	21.8	23.4	23.2	57	42	68	18.0	17.4	18.4
1951	Irrigation	16.6	18.4	16.3	56	73	67	16.4	16.7	16.6
	No irrigation	18.5	17.3	15.9	70	69	66	17.1	17.3	16.3
Mean (4 years)	Irrigation	21.5	21.7	19.5	61	73	71	16.3	16.8	16.7
	No irrigation	20.1	20.8	20.1	64	69	72	16.8	16.1	16.6
Standard Error of the difference between means ("Students'" paired variates)		1.71	0.79	1.27	12.1	3.6	7.1	0.28	0.83	0.10

[1] 100 percent stand = One beet plant per foot of row in 20-inch rows.

Table 3.—Comparison of the Surface and Ridge-Cover Methods of Planting Sugar Beets. 1948-1951.

Planting Date	Planting Method	Yield of Roots (tons per acre)	Harvest stand (percent)
	Mean Four Year Period (1948-1951)[1]		
Early	Surface	21.3	67
	Ridge-cover	20.8	62
	Standard Error[2]	0.94	8.1
Medium	Surface	21.6	82
	Ridge-cover	21.2	71
	Standard Error[2]	0.39	10.3
Late	Surface	20.4	78
	Ridge-cover	19.8	72
	Standard Error[2]	0.65	4.5

[1] Data represents mean yields of combined plots irrigated and not irrigated for emergence.

[2] Standard Error of the difference between the two means by method of "Students" paired variates.

Summary

Experiments dealing with planting date, pre-emergence irrigation and planting method were conducted during a six-year period (1948-1953) at the Scotts Bluff Experiment Station in western Nebraska.

Sugar beets planted April 2 and April 16 produced about the same mean yields over the six-year period, but a two ton per acre reduction in yield occurred where planting was delayed until May 2.

During the six-year period as a whole, yields on plots irrigated for emergence were the same as on plots not irrigated at any one planting date. During individual years, however, the crop benefited or suffered significantly from the early irrigation. Irrigated beets emerged earlier than non-irrigated beets, and the occurrence or absence of severe frost at time of emergence influenced stands and yields accordingly.

Poorest stands in the tests were associated most often with early planting and irrigation for emergence. The yields obtained, however, indicate that early planting compensated to some extent for loss in stand.

Sucrose percentage was not affected by the treatments used.

Yield trends under the ridge-cover method of planting were similar to those under the surface method, but inferior stands were associated with ridge-cover planting. Climatic conditions, physical structure of ridges, and techniques used in removing them were problems associated with ridge-cover planting of sugar beets.

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Determination of Saponins in Refined Beet Sugars

H. G. Walker, Jr.[1]

The flocculent precipitate which sometimes forms in acidified sirups made from either beet or cane sugars has created a problem for both the bottling and the refined-sugar-producing industries. The floc from beet sugar is composed of sugar-beet saponins, fat, colloidal coloring matter, and inorganic materials (1, 4)[2]. The acid-insoluble beet saponins appear to act primarily as scavenging agents, adsorbing other colloidal matter in the sirups. In this laboratory, the nature of the cane floc has not been investigated, other than to ascertain that it does not contain saponin, so that the work to be described applies only to beet sugars.

At present there is no satisfactory small-scale, rapid method for predicting the floc performance of a refined beet sugar. A gravimetric floc determination reported by Eis, Clark, McGinnis, and Alston (1) requires about five pounds of sugar and a minimum analysis time of about six to eight hours. A qualitative analysis for total floc is frequently used for control work: heated, acidified sirups are scored visually for floc content after standing for varying periods of time, usually 24 hours (2).

This paper reports the development of a method suitable for control work which is capable of estimating the few parts per million of saponins in refined beet sugars. The analysis measures the saponins and not total floc, because, in our experience, no additional floc is produced in sugar sirups from which saponin has been removed. The saponins are removed from an acidified sirup by filtration and are determined colorimetrically in glacial acetic acid with a reagent composed of 10 percent antimony pentachloride in chloroform. The elapsed time for an analysis is about two hours.

Reagents.

Chloroform: Reagent grade.

Glacial acetic acid: Reagent grade.

Antimony pentachloride: Reagent grade, as purchased, usually contains too much free chlorine to produce color with saponin (2). Vacuum distillation in an all-glass system gives a purified product satisfactory for color development. A suitable reagent can also be made by adding up to 10 mole percent of $SbCl_3$ or by blowing dry air through commercial antimony pentachloride (2).

A 10 percent (V/V) solution of $SbCl_5$ in chloroform is prepared. The reagent should be handled in a fume hood.

Standard Sugar Beet Saponin. This reference material can be isolated from factory juices or lime muds (1, 4). A solution containing 0.04 mg. of standard per ml. of glacial acetic acid makes a suitable colorimetric standard and appears to keep well in a glass-stoppered flask.

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[2] Numbers in parentheses refer to literature cited.

Procedure. Dissolve 125 gm. of refined beet sugar in 250 ml. of water and add 2.5 ml. of concentrated HCl. Add 3.75 gm. of filter aid to the solution and filter with suction through a small Buchner funnel (6 cm.) using Whatman[a] No. 5 paper precoated with 1.25 gm. of Celite analytical filter-aid. Wash the sides of the funnel and the filtered material portionwise with acidified wash water until the wash contains no more sucrose. Dry the funnel and contents in an oven for 20 minutes at 105°. Elute saponin from filter-aid with about 8 ml. of hot glacial acetic acid, collecting the eluate in a 10-ml. beaker. If necessary the acetic acid solution can be refiltered with suction through a Whatman No. 50 paper on a small funnel, collecting the clear filtrate in a graduated tube or a 10-ml. volumetric flask. After making to definite volume (as necessary) with acetic acid, pipette a 1-ml. aliquot into a colorimeter tube (19 x 105 mm.) and add exactly 7 ml. of antimony pentachloride reagent. Shake tube to mix, stopper tightly, and allow to stand at room temperature. After 10 minutes, read the absorbance at 535 $m\mu$ on a colorimeter. Calculate the mg. of saponins in the unknown sample from the absorbance of the known standard saponin reagent and a reagent blank determined at the same time. The antimony pentachloride hydrolyzes enough in moist air to coat glassware badly unless it is rinsed immediately after use with chloroform. Although the reagent attacks rubber and cork stoppers, rubber stoppers covered with Teflon tape are satisfactory. Silicone grease is a suitable stopcock lubricant for the reagent dispensing buret.

Discussion and Results

The method is based on the reaction of sugar beet saponins with antimony pentachloride to produce a red-colored camplex with an absorption maximum at 535 $m\mu$, determined on a Beckman model B spectrophotometer. Sucrose and antimony pentachloride also react to give a brownish complex with no maximum in the visible spectrum. Since, however, the sugar complex could contribute background absorbance for the saponin determination, it must be completely washed out of the saponin sample prior to color development. Fatty material in refined beet sugar does not seem to interfere, because floc samples, with or without washing by fat solvents, give identical saponin values.

Color formation depends on several variables. The red complex represents an intermediate stage of reaction, because, after long standing, the solutions become a grayish blue. Heating increases the formation rate and intensity of the red color; however, it also accelerates the fading process. When antimony pentachloride reagent is added to an acetic acid solution of saponin, enough heat is evolved to produce a color in 10 minutes at room temperature which is essentially stable for another 35 minutes. Contrary to the report of Steinle and Kahlenberg (3), light is not necessary for development of the color.

Figure 1 shows that there is a satisfactory straight-line relationship between saponin concentration and absorbance at 535 $m\mu$ for saponin values likely to be encountered in this analysis.

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[a] Mention of trade names does not imply endorsement by the Department of Agriculture over others of a similar nature not mentioned.

Tables 1 and 2 indicate that minor variations in the concentrations of antimony pentachloride in the reagent and water in the acetic acid-saponin eluate will not have a serious effect on the saponin values obtained

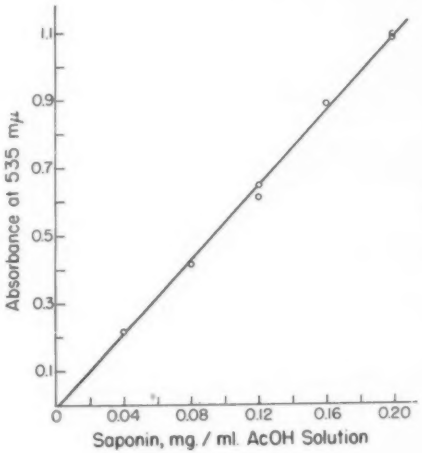

Figure 1.

Table 1.—Effect of $SbCl_5$ Concentration on Blank and Absorbance;[1] Saponin Concentration Constant.

$SbCl_5$ Concentration (%, V/V, in chloroform)	Absorbance	
	Blank	Saponin
5	0.028	0.135
10	.021	.512
15	.020	.505
20	.024	.495
25	.024	.445

[1] All absorbances are read at 535 mμ after color development has proceeded 10 minutes at room temperature.

Table 2.—Effect of Water in Saponin Acetic Acid Solution; Saponin Concentration Constant.

H_2O conc., vol. % in saponin-ACOH soln.	Absorbance[1]
0	0.459
10	.442
15	.402
20	.790[2]

[1] All absorbances are read at 535 mμ after color development has proceeded 10 minutes at room temperature.

[2] Turbid solution formed.

from the absorbance figures. The conditions outlined in the procedural section, therefore, represent an adequate way to measure beet saponins after isolation from beet sugar.

The quantitative isolution of beet saponin from the sugar sample is the most difficult problem encountered in the analysis. Clearly the task of separating and measuring an impurity present to the extent of a few parts per million is not easy. Solvent elution, distribution, or ion exchange methods were not satisfactory. It was found that filtration on sintered glass filters (1) was slower and no more efficient for floc removal than filter paper with filter aid. Table 3 shows that recovery of saponin is essentially quantitative from water, but that some loss occurs when 33 percent sirups are used.

Table 3.—Saponin Recovery from Acid Solutions, 250 ml.

Sugar (gm.)	Saponin added (mg.)	Saponin recovered (%)
0	0.20	98
0	.40	99
0	.60	93
0	.80	101
0	1.00	95
125	0.10	85, 63
125	.20	87, 63
125	.30	77, 75, 72
125	.40	76
125	.50	90, 88, 87
125	1.00	84, 83

Since, however, a reasonably constant amount of saponin can be recovered from the sirups at the various levels of saponin concentration, a correction factor could be determined, if necessary, to increase the accuracy of the method. Table 4 shows that replication of saponin values on a single sample of sugar is good, and that added amounts of saponin can be recovered from the sample. Table 5 lists the saponin analyses on a number of refined beet sugars. For comparison, floc values determined by the Spreckels method are included for some of the samples.

Table 4.—Replicate Analyses and Recovery of Added Saponin from a Beet Sugar Sample.

Saponin added (mg.)	Saponin found (mg.)
0	0.50, .56, .52 .53, .52, .47 .46, .52, .56, .52
.5	1.05, 0.95[1]
1.00	1.23, 1.28, 1.31

[1] Sample washed with chloroform before elution.

Table 5.—Saponin in Refined Beet Sugar Samples

Sample No.	p.p.m.	Spreckels analysis, p.p.m.
1	3.2	3, 4, 2
2	4.3	8
3	2.8	3
4	2.3	3
5	2.1	3
6	2.1	3
7	2.3	
8	5.4	
9	1.1	
10	3.6, 3.9, 1.9[1]	

[1] Sirup for analysis not acidified with HCl.

In general, the results obtained by this method show lower floc values because the oil content of the floc and other solids in the sirups are included in the gravimetric measurement but only the saponin is measured in the colorimetric method.

Although floc determination by the described procedure is not as accurate as might be desired, our limited experience has indicated a good correlation between floc content by visual estimation and by saponin analysis. The method should meet the needs for control work on floc, since large numbers of samples can be run easily in a fairly short time.

The author wishes to acknowledge the technical assistance of Roberta Erlandsen and to thank Dr. R. A. McGinnis of the Spreckels Sugar Company for supplying representative samples of beet sugars with the corresponding floc analyses.

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The Effect of Various Impurities on the Crystallization of Sucrose

GUY RORABAUGH AND LLOYD W. NORMAN[1]

Introduction

The physical phenomena which control the rate of crystallization of sucrose from solution are not well defined. Although much work has been done on the subjects (1)[2] sucrose-salt solutions, (2) sucrose-non-sucrose solutions, (3) and real massecuites (4) indications are that the Noyes-Whitney-Nernst equation, which assumes that diffusion of the sucrose molecules to the crystal surface is the rate controlling mechanism, is not valid. Rather it is shown, that the rate closely approximates that of a unimolecular reaction, particularly when sucrose activities are utilized rather than concentrations.

The effect of purity upon the crystallization of sucrose from beet-house sirups has been clearly shown by Nees and Hungerford (5). Seasonal and district variations are indicated by the same work and also by the later work of Hungerford (6). It is the general belief, that all impurities associated with natural beet sirups impede crystallization, although Suzuki (7) has obtained evidence (not well verified) of a beneficial action by traces of manganese sulfate.

Van Hook and Shields (2) have separated the impurities found in natural sirups into two groups: the electrolytes or ash portion and the non-electrolytes or organic non-sugar constituents. In each case, they have correlated satisfactorily their own data as well as that of other workers (8) (9) with the activity concept of crystallization behavior.

Data on the relative inhibitory effect of impurities normally found in beet sirups are rather meager. Amagassa has reported on several salts, including Na_2CO_3, K_2CO_3, NaCl, KCl, $NaC_2H_3O_2$, $KC_2H_3O_2$ and Na_2SO_4, listed in the approximate decreasing order of their effect upon crystallization (8). Other workers confirm the inhibitory power of salts, but generally do not compare their relative effects.

Of the organic non-sugars, the inhibitory power of raffinose has been given considerable attention (6, 3). Other materials on which work has been reported include invert sugar, dextrose, amino acids, and caramel (3, 10, 9). European literature contains many references to "noxious nitrogen" and "harmful nitrogen" with no definite information as to the exact role of individual nitrogenous substances as molasses formers or their effect on crystallization.

The solubility of pure sucrose in water has been determined by several workers with the data of Herzfeld (11), published in 1892, having been accepted more or less as standard, even up to the present time. However, information regarding the solubility of sucrose in solutions of the common sugar-house impurities is limited. Brown and Nees (12) have determined

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[2] Numbers in parentheses refer to literature cited.

the solubility in a wide variety of beet-house sirups. Jackson and Silsbee (13) have measured the solubility in invert sugar solutions. Shukow (14) determined the amount of sucrose which would dissolve in various salt solutions, and Kelly (15) more recently determined the equilibria of some similar ternary systems.

The desirability of segregating the relative effects of the various impurities upon the crystallization of sucrose from sugar-house sirups has long been recognized, but lack of analytical data and methods has prevented the sugar technologists from being able to control adequately, experimental conditions so that quantitative interpretation of results in terms of individual components (some of which could not even be identified) has been impossible. With the development of new analytical techniques, such as paper chromatography and ion exchange, Owens and his associates have been able to add considerably to the knowledge of the composition of beet-house sirups and juices (16, 17, 18, 19).

The present work was undertaken for the purpose of obtaining practical information regarding the relative effect of some of the more prevalent impurities found in beet sirups after carbonation upon the crystallization of sucrose. The identity and amount of these contaminants were furnished by Owens (20). Benefit resulting from determination of such relative inhibitory effect was visualized as twofold. In the first place, knowledge of which impurities cause the greatest difficulty in crystallization would allow the focusing of attention upon removal by existing, as well as supplementary, processing techniques. Also, and probably of even greater value, would be the opportunity to develop a beet breeding program aimed at eliminating or reducing the more troublesome compounds from the beet itself, since a great many of the impurities are impossible to remove in the factory with the processing methods used today. If beets can be selected to give a high sugar yield per acre and, in turn, be low in impurities that cause trouble in processing and crystallization, it follows that more sugar can be recovered per ton of beets processed and at a lower cost.

The work as developed can be divided into three separate phases. The first phase involved the use of actual beet-house massecuites which were recrystallized in the presence of known amounts of individual impurity. A second phase involved the preparation of synthetic sirups from pure sucrose and known added amounts of chosen impurities. In this case, crystallization tests were carried out both with the addition of increments of individual impurities and, also, with the elimination of such impurities. The third phase included solubility determinations of sucrose dissolved in solutions of individual impurities.

Procedures

The experimental work was carried out in a constant temperature air bath, a picture of which is shown in Figure 1.

The temperature was maintained constant by passing the air in the thermostat through slots in the false bottom, over a bank of ordinary light bulbs, through a circulating fan, and back into the main body of the thermostat. The temperature sensing element was an ordinary mercury

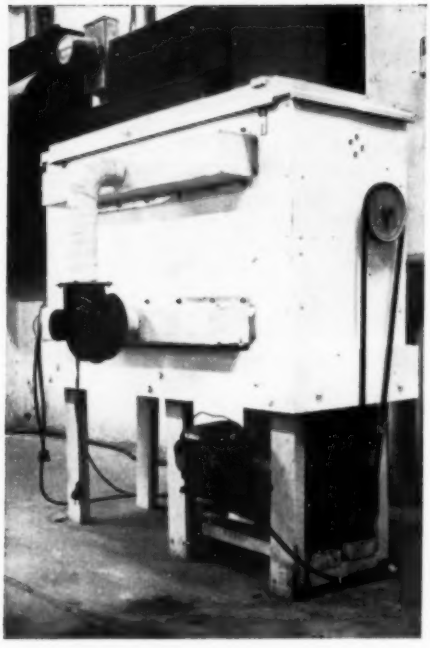

Figure 1. — Air bath for crystallization tests showing circulating fan and manifold.

thermometer and a "Thermocap" relay, which controlled the heat source. Very accurate control of temperature is possible.

The samples to be crystallized were placed in pint fruit jars held in ring adapters. This assembly was then placed in the thermostat on a pair of rubber-covered rollers which were rotated mechanically, thus turning the ring adapter and fruit jar. The jars were rotated at a speed of ⅓ R.P.M. to provide continuous gentle agitation of the sample.

In the first phase of crystallization studies reported herein, a low-raw massecuite from the Sidney, Montana, factory was employed. This massecuite had a true purity (on R.D.S. basis) of 81.76. A series of runs was conducted with this low-raw massecuite as the basic material.

A batch of the low-raw massecuite was first diluted in order to dissolve all of the crystalline sugar present. It was then evaporated under vacuum to a concentration slightly above the 1.1 supersaturation desired and finally diluted carefully with distilled water to the exact concentration as determined by the Bausch and Lomb precision refractometer. Supersaturation of 1.1 was calculated as given by McGinnis (21). Concentration of saturated beet sugar syrups at varying temperatures and purity are arbitrarily taken from Brown, et al. (22).

For each individual sample 250 grams of the base solution was weighed into a pint fruit jar and the desired impurity to be tested was added in the

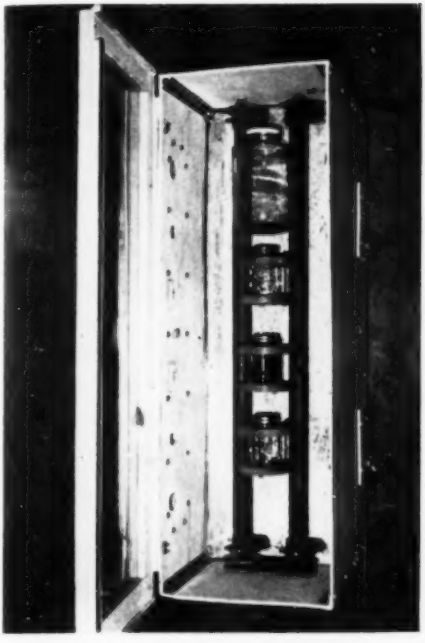

Figure 1-A.—Inside view of air bath showing rolls and adapter rings on jars.

approximate amount reported, based on its occurrence in molasses (20). After the impurities had been dissolved, the jars were fastened in the ring adapters previously described and the assembly placed in the air bath held at 120° F. The samples were held in the air bath overnight at this temperature to allow them to reach equilibrium at this temperature.

At 8:00 the following morning the samples were seeded with 75 grams of screened seed sugar (—42 + 48-mesh). Since all could not be seeded simultaneously, they were seeded individually at five-minute intervals (starting at exactly 8:00 a.m.). The air-bath temperature was arbitrarily lowered 5° F. every hour on the hour to provide a simulated crystallizer temperature pattern. The final temperature adjustment to 85° F. was made at 3:00 p.m.

At 4:00 p.m., the first seeded sample was removed and its R.D.S. determined. The remaining samples were removed and their R.D.S. determined at five-minute intervals in the order in which they were seeded. Loss in R.D.S. was taken as sugar crystallized.

In addition to the determination of crystallization after eight-hours crystallization, the samples were also allowed to remain in the air bath at room temperature for several days in order to determine total crystallization at equilibrium conditions. In these tests, all heating elements were turned off at 5:00 p.m. on the day seeded, thus allowing the temperature to drop slowly from 85° F. to room temperature (73° F.) and remain at room temperature for the rest of the test.

In the second phase of these studies, synthetic solutions of chosen impurities were employed. Two approaches to the problem were used. In the first, each individual impurity whose effect was to be studied was eliminated from one sample, with the relative increase in crystallization due to its elimination being taken as its relative inhibitory power. For the second approach, a stock solution of all chosen impurities was prepared and each individual impurity added in further quantity as it was tested, with decrease in crystallization being taken as a measure of melassigenic power. All impurities were tested in equal concentration except as noted.

Of necessity, in the series in which the impurity components were individually eliminated, each sample was prepared separately. Each impurity was present in the ratio of 1 part per 100 parts of sucrose except the leucines, which were added in the amount of 0.35 part per 100 parts of sucrose due to their limited solubility. A final sirup purity of 92.3 was employed in every case.

The impurities, sucrose, and water were accurately weighed into the pint sample jars to provide 250 grams of sirup at the concentration desired. The jars were then sealed and the solids dissolved by agitation of the jars in a hot-water bath. The samples were then cooled to about 55° C. and final minor adjustment of concentration to 1.1 supersaturation made according to R.D.S. As in the massecuite samples, the samples were allowed to remain overnight in the air bath at 120° F. Seeding temperature lowering, and final R.D.S. determination at eight hours and several days were also conducted in the same manner as before. Levels of pH were held the same by adjustment with potassium hydroxide to a pH of 9.

For the test series in which impurities were an added factor rather than an eliminated one, a stock solution of impurities was first prepared in the approximate ratio in which they have been found in beet molasses (20, 21), with the pH adjusted to 9. Samples were then prepared in pint jars from weighed amounts of stock solution, sucrose, and each individual added impurity. Dissolution of solids, concentration adjustment, and actual crystallization testing were all carried out in the same manner as before. Added impurities were all in the ratio of 1 part per 100 parts of sucrose except leucine, which was added in the ratio of 0.5 part per 100 parts sucrose. Some difficulty was encountered in obtaining complete dissolution of a few of the impurities with K_2SO_4 being the most troublesome. It was thought, however, that all impurities dissolved in the presence of sucrose in the hot-water bath. Whether or not some of the least soluble ones were precipitated out in the crystallization runs is not known.

The final phase of the present studies involved determination of the solubility of sucrose in solutions of some known impurities found in beet molasses. Solutions of the individual impurities were prepared in pint fruit jars in concentrations of 10 grams impurity plus 100 grams water, except aspartic and glutamic acids which were saturated solutions (at 30° C.). After the solutions were allowed to reach equilibrium at 30° C. an excess of + 42-mesh crystalline sugar was added. The jars were then replaced in the air bath at 30° C. and allowed to remain with constant slow

agitation for six days. The jars were then removed and R.D.S. determined in order to calculate the amount of sucrose dissolved.

Results—Phase I, Natural Sirups + Added Impurities

The effect of several chosen impurities upon crystallization from a low-raw massecuite base stock was determined by the methods previously described. The impurities chosen for the tests are shown in Table 1.

In eight-hour crystallization tests, all of these impurities significantly lowered the crystallization rate (to 1 percent level of statistical significance), except betaine.

A check of the precision of the method of running the crystallization tests was deemed an essential factor in the early runs in order to prove the usefulness of the method. In a series of tests involving three replications of seven treatments, it was found that the standard deviation among replications was 0.028 R.D.S.

This small deviation between replications and the fact that differences between treatments in the order of 0.10 R.D.S. were found significant to the 1 percent level indicates that the method should be very useful in detecting small changes in crystallization rate. It also points up the fact that extreme care must be used in preparation of samples for testing, as well as in the actual measurements involved.

Included in Table 1 is a list of the various impurities used in decreasing order of their crystallization inhibitory power. The first column shows the relationship at the approximate concentration level known to exist in cretain beet sirups. The second column gives the list on the calculated basis of equal concentrations of 1 part per 100 parts of sucrose. This was calculated by the difference in R.D.S. obtained divided by the parts of impurity per 100 parts sugar used in the test. This may not be necessarily true as it assumes the effect is linear with concentration; however, it provides a relative effect of the individual impurity.

Table 1.—Impurities Given in Decreasing Order of Their Effect Upon Crystallization.

Actual Concentration Basis	Equal Concentration Basis
1. Betaine	Calcium chloride
2. Glutamic acid	Serine
3. Aspartic acid	Calcium sulfate
4. Leucines	Aspartic acid
5. Sodium sulfate	Leucines
6. Alanine	Glutamic acid
7. Serine	Alanine
8. Calcium chloride	Sodium culfate
9. Calcium sulfate	Betaine

It is immediately apparent that the difference in basis makes a great difference in the relative ranking of the melassigenic power. Although the unit effect of betaine is the lowest of any of those impurities tested, its cumulative effect, due to its rather high concentration in beet sirups, is the greatest of any. Conversely, the two calcium salts and serine are present in such small quantities that their effect is relatively small, but if they were

present in equal concentrations to the other materials their effect would top the list.

Results—Phase II, Part 1—Synthetic Sirups with Individual Impurities Eliminated

In the second phase of the present work synthetic sirups were employed exclusively. The synthetic sirups were comprised of purse sucrose, water, and chosen impurities known to exist in beet-house sirups in significant quantities. Besides the synthetic nature of the sirups, the tests in this phase of the work also differed from the first in another important respect. Whereas impurity concentrations approximating those found naturally in beet-house sirups were employed with the natural sirups, equal weight concentrations were used with the synthetic sirups unless solubility limitations prevented. By using equal concentrations the relative melassigenic effects of the individual impurities could be compared directly.

Since the impurities to be studied in this series of tests were eliminated from the individual samples, their inhibitory effect was also eliminated. Thus, in the results of these tests the crystallization was greater for those samples which have the greater inhibitory power. In Table 2 are shown the results of this series of runs with data taken at 8-hours, 24-hours and 4-days crystallization time.

Table 2.—Synthetic Sirups With Individual Impurities Eliminated.

	8-hours Crystallization		24-hours Crystallization		4-days Crystallization	
Impurity Eliminated	R.D.S.	Percent Crystallization	R.D.S.	Percent Crystallization	R.D.S.	Percent Crystallization
Malic acid	71.66	74.00	70.62	85.71	70.50	88.09
Lactic acid	71.65	75.90	70.62	85.71	70.49	88.27
K_2SO_4	—	—	70.46	89.15	70.42	89.91
Betaine	71.58	77.14	70.49	88.19	70.37	90.56
Leucines	—	—	70.37	90.75	70.32	91.70
Glutamic acid	71.41	80.20	70.40	89.92	70.27	92.48
Aspartic acid	71.44	79.01	70.37	90.56	70.26	92.71
P.C.A.	71.41	80.48	70.37	90.49	70.25	92.84
KCl	—	—	70.28	92.50	70.72	93.63
Alanine	71.36	81.57	70.32	91.59	70.14	95.06

NOTE: All impurities present in concentration of 1 part per 100 parts of sucrose except leucines which, due to low solubility were added in concentration of 0.35 part per 100 parts sucrose.

A plot of crystallization time versus percent crystallization realized is given in Figure 2 for the fastest and slowest crystallizing sirups, as well as two intermediate ones. The curves show that after about 2½ days' time little additional crystallization takes place and, therefore, any crystallization time greater than this could be considered essentially at equilibrium conditions. The following list shows the impurities in order of the decreasing effect upon the crystallization.

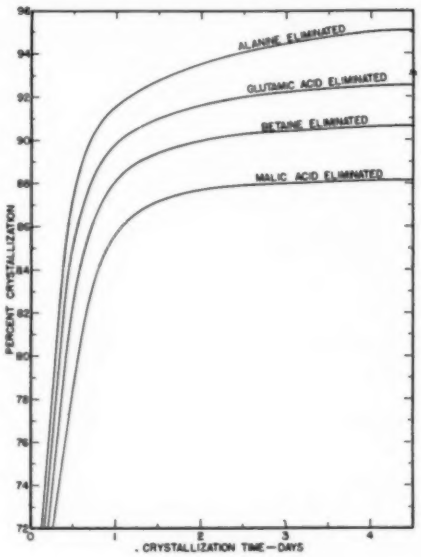

Figure 2.—Percent crystallization versus time.

Table 3.—Some Beet-House Impurities in Order of Their Decreasing Effect Upon Crystallization of Sucrose From Synthetic Sirups.

1. Leucines[1]	6. Glutamic acid
2. Alanine	7. Betaine
3. Potassium chloride	8. Potassium sulfate
4. P.C.A.	9. Lactic acid
5. Aspartic acid	10. Malic acid

[1] Leucines corrected to 1 part per 100 sucrose. Ranked between glutamic acid and betaine on .35/100 as tested.

A comparison of this table with the results obtained for both eight-hours crystallization and one-day crystallization will show only minor changes in the order between impurities which have been very close to the same effect. Similarity of the results to those obtained with natural sirups, as given in Table 1, will also be noted.

In order to assign a specific value in terms of the amount of sucrose lost (held in solution) per unit weight of impurity, we assumed that malic acid has little or no effect upon the crystallization; actually, it had the least effect. Thus, if the difference between the effect of malic acid and each of the other impurities is determined, an arbitrary measure of the amount of sucrose lost will be obtained. In effect it could then be said that, if malic acid could be exchanged for the impurity involved, an increased recovery of sucrose could be realized. Under the condition of these tests and the assumption above, the following increase in sugar recovery could be realized in pounds sucrose per pound of impurity per 100 pounds original sucrose in solution before crystallization.

Table 4.—Increased Sucrose Recovery Obtained by Exchange of Malic Acid For Other Impurities.

Impurity	Pounds Additional Sucrose
Lactic acid	0.07
Potassium sulfate	0.60
Betaine	0.62
Glutamic acid	1.28
P.C.A.	1.32
Aspartic acid	1.42
Alanine	2.55
Leucines[1]	3.43

[1] corrected to 1 part/100 parts sucrose.

A comparison of this nature shows up the practical significance of the difference in effect of the various impurities. For instance, those impurities which have the greatest effect per unit of weight are severalfold more troublesome than those on the lower end of the group.

Phase II, Part 2—Synthetic Sirups with Added Impurities

To provide a better basis of calculations of the amount of sucrose held in solution by a given weight of impurity and to make further comparison of the relative effect of various impurities upon the crystallization of sucrose, additional tests were conducted which consisted of crystallization runs in which known amounts of individual impurities were added to a synthetic stock sirup of known composition. Not only could the relative effect of each impurity be determined, as in the tests described earlier, but also the specific effect of each could be calculated in comparison to crystallization from the basic stock sirup.

In these tests, since the impurities were added to a basic sirup, the effect will be a lowering of the crystallization. The relative crystallization for 4-day and 6-day tests is presented in Table 5.

Table 5.—Relative Crystallization Obtained With the Addition of Selected Impurities to a Synthetic Base Sirup.

Impurity Added	4-days Crystallization		6-days Crystallization	
	R.D.S.	Percent Cryst.	R.D.S.	Percent Cryst.
K_2CO_3	71.07	60.82	70.98	62.28
KCl	70.88	63.88	70.77	65.64
Leucine	70.68	67.08	—	—
Gltuamic acid	70.75	65.96	70.67	67.24
Alanine	70.74	66.12	70.64	67.69
Aspartic acid	70.73	66.28	—	—
P.C.A.	70.67	67.24	70.57	68.81
Lactic acid	70.64	67.69	—	—
Malic acid	70.62	68.01	70.52	69.59
Betaine	70.58	68.65	70.49	70.04
K_2SO_4	70.54	69.27	70.46	70.52
Check	70.54	69.27	70.48	70.20

NOTE: All added impurities present in the amount of 1 part per 100 parts sucrose except leucine, which was added in the amount of 0.5 part per 100 parts sucrose.

As noted in the previous series, the crystallization had nearly reached equilibrium conditions after four days. Although there was a measurable drop in R.D.S. between four and six days, the amount was small and virtually constant for all samples, being from 0.08 to 0.11 R.D.S. units. No differences at all could be measured, for a few chosen samples, between the six-day readings and readings after 10-days crystallization.

Some changes in ranking will be noted from Table 5 in comparison with previous results. Potassium carbonate, which was not included in other tests, proved to be the worst offender of all. Alanine dropped a couple of notches, and P.C.A. and glutamic acid more or less "swapped" positions. Lactic and malic acids, which were previously the least inhibitive, climbed above betaine and potassium sulfate, although all of there were relatively low. Nevertheless, the same general pattern persists in that the previously high ranking impurities still ranked high and the low ones still low.

The "check" sample included in this series of tests provides a good basis upon which to calculate the absolute amount of sugar lost (held in solution) by a given quantity of each impurity, since the "check" sample consisted of the basic sirup only without added impurity corrected to the same R.D.S. and purity as the other solutions. The difference between the absolute quantity of sugar crystallized from each sample and that crystallized from the "check" sample will give the quantity of sugar lost per unit of impurity added per 100 units of sucrose in the original sirup at the purity employed (86.0 in original sirup). It should be recognized that, since the effect upon crystallization is not directly proportional to concentration, other concentrations at other purities would not necessarily give the same values. Yet, as already pointed out, such figures will be more significant in estimation of actual dollars and cents loss and will show clearly the area in which elimination of specific impurities, regardless of method of elimination, will be most profitable.

In Table 6 are presented the relative quantities of sugar lost in terms of pounds per pound of impurity per 100 pounds sucrose originally present.

Table 6.—Pounds of Sucrose Lost Per Pound of Individual Impurity Per 100 Pounds of Sucrose Originally Present.

Impurity	Pounds Sucrose Lost
Potassium carbonate	3.70
Potassium chloride	2.36
Leucine[1]	1.94
Glutamic acid	1.45
Alanine	1.38
Aspartic acid	1.31
P.C.A.	0.89
Lactic acid	0.69
Malic acid	0.55
Betaine	0.27
Potassium sulfate	—

[1] Corrected to concentration of 1 part per 100 parts sucrose.

Again, it will be noted that the impurities having the greatest effect upon crystallization hold several times as much sucrose in solution as those which have the least effect. If we now combine the additional factor of concentration with the sucrose holding power, as given above, while keeping in mind the fact that the effect need not be directly proportional to concentration, a good indication is obtained of the total melassigenic power of some of the major impurity constituents found in beet-house sirups.

In Table 7, is given the average composition for molasses as furnished by Owens (20), and the total sugar which would be held in solution by such quantity of impurity based upon the data given in Table 6.

Table 7.—Total Sugar Lost in Molasses by Crystallization Inhibiting Effect of Some Chosen Impurities.

Impurity	Average Occurrence in Molasses (grams/100 grams Sucrose)	Total Sugar Held by Impurity (pounds/100 pounds Sucrose)
Potassium carbonate[1]	8.00	29.60
Potassium chloride[2]	8.00	18.88
Leucines	0.35	0.68
Glutamic acid	1.25	1.81
Alanine	0.85	1.17
Aspartic acid	0.90	1.18
P.C.A.	5.50	4.90
Lactic acid	2.80	1.93
Malic acid	1.00	0.55
Betaine	9.00	2.43
Potassium sulfate[2]	3.70	—
TOTAL:	41.35	63.13

$$\text{Purity} = \frac{63.13}{104.48} \times 100 = 60.42$$

[1] Not reported by Owens. Taken here as equal to chloride content. Carbonate reported elsewhere (21) as over twice the chloride.

[2] Chloride and sulfate content as the potassium salt.

Table 7 provides food for thought along several different lines. In the first place, it will be seen that over 75 percent of the total sugar held in molasses is there as a result of two of the ash components, potassium carbonate and potassium chloride. Not only are these compounds the two top inhibitors of sucrose crystallization, but they are also high on the list of average occurrence. Thus, their combined effect becomes a large fraction of the total effect of all impurities.

We have found very little in the literature on the concentration of carbonate in molasses or other beet-house sirups. In McGinnis' text on "Sugar Beet Technology" (p. 439) a typical analysis for California molasses shows carbonate content over twice that of chloride. We have arbitrarily taken the carbonate content as equal to the chloride. Therefore, if the carbonate content were doubled, it alone would account for over 60 percent of the sucrose loss. Regardless, however, it appears that any measures which can be taken to lower the carbonate and chloride content of beet sirups

would be of great benefit. Following the carbonate and chloride salts, the amino acids and betaine are next most troublesome. Next in line come the non-nitrogenous organic acids and, last, with little or no apparent harmful effect, will come the sulfate salts.

Of the nitrogen compounds, P.C.A. plus glutamic acid have the greatest total effect upon crystallization primarily because of their relatively high concentration. Their combined effect is over 10 percent of the total and, thus, comprises a significant portion of the sugar loss.

It is fortunate, indeed, that betaine has a relatively small effect upon crystallization. Betaine provides nearly 22 percent of the total impurities, as given in Table 7, yet less than 4 percent of the sucrose loss is attributed to the compound. On the other hand, the leucines and alanine are relatively high in the ranking of inhibitory power, but their limited concentration makes them a small factor in over-all molasses forming tendency.

Malic and lactic acids are present in molasses in significant quantities, but they do not contribute greatly to sucrose loss since they are not strongly melassigenic.

Potassium sulfate, at least in these tests, is in a class by itself. Little effect upon crystallization was indicated.

Phase III—Solubility of Sucrose in Some Solutions of Selected Impurities.

In this final phase of the present studies, a brief series of tests were conducted in which the solubility of sucrose was determined in some solutions of several of the impurities studied in the crystallization tests. These tests were conducted at a constant temperaure of 30° C. in the same equipment used in the crystallization studies. Table 8 gives the results of the solubility tests.

Table 8.—Solubility of Sucrose in Solutions of Some Selected Impurities.

Impurity	Final R.D.S.	Solubility grams Sucrose/100 grams H_2O	R.D.S. of Impurity Solution
P.C.A.	74.12	276.4	8.96
Betaine	68.14	203.9	7.83
Malic acid	73.45	266.6	7.32
Alanine	69.08	213.4	10.72
Glutamic acid[1]	68.58	208.3	1.34
Aspartic acid[1]	68.78	210.3	0.80
Potassium carbonate	70.88	233.4	10.28
Potassium chloride	69.58	218.7	8.33
Potassium sulfate	68.79	210.4	7.41
Sodium carbonate	71.04	253.3	13.10
Sodium chloride	69.67	219.7	10.65
Sodium sulfate	68.49	207.4	9.10
Check	68.52	217.7	—

[1] Saturated solution (at 30° C.) employed for Aspartic and Glutamic acids.

The results of the solubility tests seem to point out several things. In the first place, the results should be fairly accurate, since the solubility of sucrose in water (the "check" sample) is in good agreement with the value

of Herzfeld (11). The data for solubility in salt solutions checks the crystallization results in that the order of effect of carbonate-chloride-sulfate corresponds to the same order of effect found in the crystallization tests. These tests also provide a comparison of potassium with sodium and indicate that sodium should be more harmful than potassium. This relationship has been given previously in the literature (8).

Betaine is shown to hold a low amount of sucrose in solution, also in agreement with the crystallization results reported herein. The effect of the amino acids does not check well with the crystallization results, and an even greater discrepancy is noted for malic acid, which allowed the second greatest solubility of sucrose.

A recent paper by Wiklund (23) suggests the presence of molecular compounds between sucrose and salts which, if his hypothesis is valid, would help explain some of the differences in solubility not following the results obtained by crystallization.

The last column in Table 8, giving the R.D.S. of the impurity solution before the addition of sugar, is included to show the R.D.S. variations of the various materials. R.D.S. for the impurity solutions (except glutamic and aspartic acids) should have been 9.09, since 10 grams were dissolved in 100 grams H_2O. If we take the greatest variation from this 9.09 figure we find sodium carbonate to be 4.01 higher and malic acid to be 1.76 lower. At a total solids of about 220 grams/100 grams H_2O this variation would amount to 0.9 to 1.8 percent deviation.

Discussion of Results

In general, the order in which some of the common beet-sirup impurities affect crystallization was found to be as follows:

1. Carbonate and chloride salts
2. Amino acids
3. Betaine and non-nitrogenous organic acids
4. Sulfate salts

Some variation in position among individuals in these groups was encountered, but the pattern of groupings, as shown, was rather consistent throughout these tests.

It is unfortunate, indeed, that chlorides and carbonates are so strongly melassigenic, since they are present in relatively large concentrations in the beet sirups and, therefore, account for a large fraction of the total sugar lost in molasses. Yet, on the other hand, it is fortunate that betaine and the sulfate salts, also present in rather large quantities, are at the bottom of the list.

From the data which we have gathered, it would appear that the most fertile ground for improvement of the crystallization characteristics lies in the elimination of carbonate and chloride salts with lowering of P.C.A. and glutamic acid the second most likely point of attack. The former pair, which appear to account for over ¾ of the total melassigenic power, should warrant the most concentrated efforts. The latter two also rate some attention, since they contribute over 10 percent to the total.

The case against P.C.A. and glutamic acid is a two-edged one, since not only are they major contributors to sugar loss in molasses, but also decomposition of glutamine, the amide from which they originate, causes processing difficulties through lowering of buffering capacity of juice and lowering of alkalinity. At the present time no method of elimination of glutamine by processing techniques is usable in the sugar factory, thus its elimination is also in the hands of the beet breeder.

Since glutamine in Steffen waste (in the form of P.C.A. and glutamic acid) has some value as a raw material for the production of monosodium glutamate, its presence in beet juices is not a total loss. It is problematic whether lowering the amount present would increase ease of processing to a sufficient extent to offset its value in Steffen waste. On the other hand, it would seem wise to think twice before purposely increasing the glutamine content of beets merely to increase the amount recoverable from Steffen waste.

Our work has indicated that, aside from carbonate and chloride salts and the decomposition products of glutamine, the remainder of the impurities examined present in molasses account for less than 15 percent of the sugar loss. It would seem logical, then, to concentrate first on elimination of the aforementioned compounds and disregard, for the present at least, the rest of the impurities.

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Sugar Cooler—Tubular, Water Cooled

A. L. Cooper[1]

Development of apparatus for cooling granulated sugar to improve its storage, transport, and packaging qualities has received much attention from the industry in recent years. It has long been recognized that relatively cool sugar is responsible for better weights and uniformity of packages. Hot sugar, when stored in large bulk bins, seems to have a thermal driving force which causes the residual moisture to migrate to cooler zones, resulting in hard cores particularly in the lower one-third of the bins, and excessive moisture content adjacent to the walls and floor. Some of the earlier bulk storage bins, such as those at the Sidney, Montana plant, have hollow walls through which warm air is circulated to offset this condition. This practice results in a slight increase in sugar color over a period of time if an excessive amount of heat is used.

The bulk bins at Carlton, California were built with solid walls with the thought that climatic conditions in the Imperial Valley would not require any special preventive measures, other than the dehumidification of the scavenging air introduced above the sugar. Such is not the case and observations indicate that difficulties with bulk sugar stored at Carlton would be reduced to a satisfactory minimum if the sugar could be sent to the bins at a temperature approximately equal to the mean ambient. This reasoning is well substantiated by experience with sacked sugar in the warehouse where it quickly cools to the temperature of the surrounding air. No means are used for controlling relative humidity or temperature in the warehouse.

It was this need that prompted the development of a continuous sugar cooler. After investigating several types, it was decided to start experimental work on a tubular, water cooled design.

Experimental Units

The first experimental tube-unit consisted of a 3-inch O.D., 16-gauge, brass tube 20-feet long mounted vertically in a 4-inch pipe water jacket. Water at 15°C. was pumped through the jacket at 10.5 g.p.m. with a ½-inch Jabsco pump. The lower end of the brass tube was closed and hot sugar introduced at the top until full, after which it was allowed to flow continuously as a column through the tube at a rate of approximately 10 lbs./min. This flow rate was governed by an .8-inch diameter orifice at the bottom. Results were unsatisfactory as the laminar flow resulted in a hot core. To prove this point, a dumy 1¼-inch O.D. brass tube was installed concentrically within the 3-inch tube and, although the sugar retention time was decreased, there was a greater temperature drop for the same flow rate. Smaller and lager orifices were tried and corresponding flow rates obtained, but it was found that the original size, i.e., .8-inch diameter gave the best overall results. The average sugar temperature drop was 8.7°C.

The final arrangement was to mount a 1½-inch O.D. brass tube concentrically within the 3-inch tube and circulate water through it, as well as around the 3-inch tube. This resulted in an annular sugar space between the two water cooled surfaces, whose inside diameter was 1½-inch and outside the diameter 2⅞-inch, or a ring thickness of 11/16 inch.

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The following are data obtained in nine tests:

Sugar temperature in	55.2°C.
Sugar temperature out	35.5°C.
Water temperature in	14.6°C.
Water temperature out	15.3°C.
Cooling water	10.5 g.p.m.
Sugar flow	9.72 lbs./min.
Sugar retention time in tube	3.7 min.
Cooling surface	22.6 sq. ft.
Heat transfer rate	6260 Btu/hr.
Coefficient of heat transfer	5.23 Btu/sq. ft./°F./hr.

Design and Operation of Full-Scale Cooler

The complete assembly of a full-scale unit consists essentially of the cooler and a bucket elevator, each having a maximum capacity of 50,000 lbs. granulated sugar per hour. The cooler has an over-all diameter of approximately 4.5 feet. The cooler and the elevator require a relatively small amount of floor space, and 45-50 feet vertical distance. This arrangement lends itself very well to adaptation in our existing plants (extending from the ground floor through the pan floor) and permits outage of the cooler-elevator unit without shut-down of other machinery.

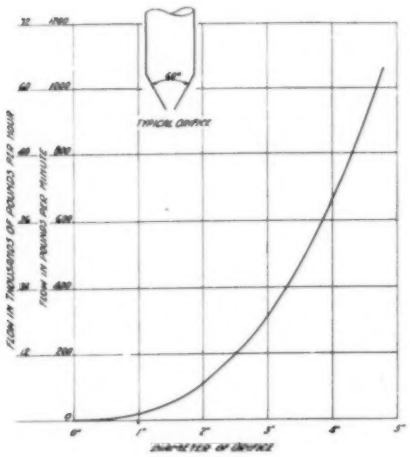

Figure 1.—Flow of sugar through orifice.

The graph in Figure 1 shows the relationship between orifice diameter and rate of discharge of dry granulated sugar. Actual flow rates of several sizes of orifices were obtained, from which was derived the formula:

$$F = KD^{2.6}$$

where K = 18.13 — a constant for fine granulated sugar.
D = Diameter of orifice in inches.
F = Flow — pounds of sugar/minute.

This formula applies only to an orifice having a 60° conical approach lcoated at the bottom of a tube, and has been found to be less than 3 percent in error for fine granulated sugar. It is an essential part of the cooler since a constant rate of through-put for each tube-unit is desirable. All tube-units that are in service are full of sugar and discharge at an average rate of 9.7 lbs./min./tube, except those on the fringe of the conical pile of sugar in the inlet chamber. Through-put of the cooler is accomplished automatically because the angle of repose of the sugar in the inlet chamber covers only a sufficient number of tubes to accommodate the quantity entering. In other words, if the cooler is operating at less than maximum capacity, some of the outer tubes remain empty. This results in a fairly uniform sugar discharge temperature regardless of through-put, other things being equal.

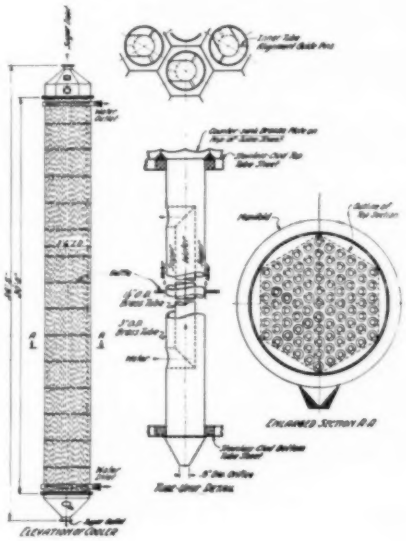

Figure 2.—Detail of sugar cooler.

As mentioned before, the reason for the "Tube within a tube" arrangement is that sugar is a very poor conductor of heat and has to pass between cooling surfaces in a relatively thin layer. (See Figure 2). Obviously, tube-units consisting of a single tube only, and of much smaller diameter, could be utilized and accomplish the same results but the discharge orifice would become so small that there would be plugging by very small lumps. The counter-sunk bronze plate resting on the top tube sheet makes the cooler self-cleaning.

Excessive clearance is provided where the tubes pass through the baffles and there are no "passes" as such. The function of these baffles is to cause a thorough mixture of the coolant as it passes upward and provide sufficient coolant pressure drop to induce flow through the 1½ inch tubes.

Salient Features of the Cooler are:

Maximum rated capacity 50,000 lb./hour.
Cooling surface—86 tube-units 2924 sq. ft.
Sugar contained in tubes at maximum capacity 4235 pounds.
Sugar retention time 5.0 minutes.
Coefficient of heat transfer 5.2 Btu/sq. ft./°F./hour.

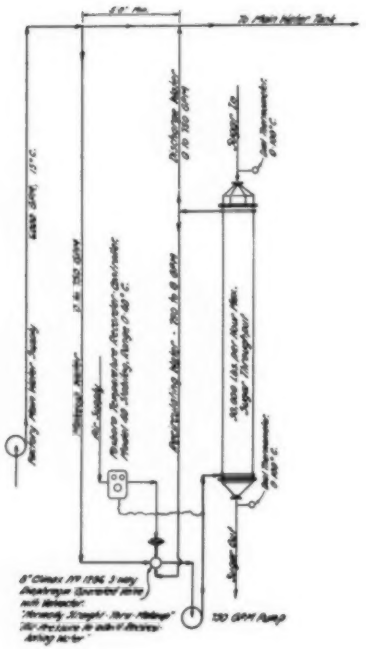

Figure 3.—Torrington sugar cooler.

Two coolers were built and installed, one at Torrington in 1951 and the second at Carlton in 1952. Main water supply temperature at Torrington, about 15°C., was colder than necessary for use as a coolant and, to prevent the tubes from sweating, it was necessary to install a temperature controller, connected as shown in Figure 3. When the 3-way valve is arranged to allow recirculation of the cooling water at any desired temperature and the controller is adjusted to 30°C., the resultant 15°C. over-all sugar temperature drop (from 55°C. to 40°C.) through the cooler, elevators, and packaging equipment is found to be sufficient. The cooling water is returned to the main supply header and no water is lost. There are no bulk bins at that plant.

The Carlton cooler requires refrigeration because the main water supply temperature ranges from 20 to 30°C. A 60 ton, closed circuit refrigeration system provides cooling water at 17°C. constant temperature. Because of the high humidity in the Imperial Valley, a size CHK Lectrodryer activat-

ed alumina dehumidifier, capable of supplying 250 c.f.m. air having a —14°C. Dew Point under normal atmospheric conditions, is used for purging the cooler. This air is introduced at the top of the cooler and carried down through three tubes, thence upward through the vacant tubes and out the sugar inlet pipe counterflow to the sugar stream to carry off the moisture released by the hot sugar. This is necessary to prevent the tubes from sweating, and functions quite well, except when atmospheric relative humidities of 50 to 65 percent, or higher, are encountered at dry bulb temperatures of 160°F and above. Under such conditions, the dehumidifier is overloaded and incapable of supplying sufficiently dry air, and it becomes difficult to maintain the cooler in operation. Air to the Lectrodryer passes through efficient filters and the Precipitron that provides the sugar dryer with cleaned outside air. For best bulk storage results the cooler should be capable of reducing the temperature of the sugar to 29 or 30°C. which would correspond closely to the 24-hour mean ambient temperature in the Imperial Valley. The system has ample capacity to reach such a temperature, but tube sweating occurs when attempting to operate at much below 35°C. Figure 4 is a typical 24-hour recording of temperatures of sugar to and from the cooler, and of the coolant.

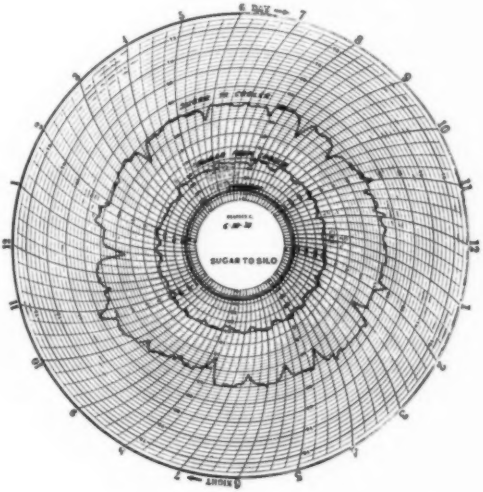

Figure 4.

It is our conclusion that the cooler, using main supply water controlled as at Torrington and purged with dehumidified air, would function satisfactorily under almost all atmospheric conditions except those that are encountered in the Imperial Valley the latter part of July and August.

Computing Supersaturation for Sucrose Solutions

EDGAR B. COLE[1]

In this paper the equation for "The Solubility at High Temperatures of Pure Sucrose in Water," by Taylor (1) is plotted against the "Boiling Point Elevation Equation for Pure Sucrose," by Spengler (2). The resulting data are used to construct tables and charts which we have found to be valuable in the study of the supersaturation of sucrose solutions. This discussion will be confined to the study of sucrose solutions of 100 percent purity. However, the problems encountered in correcting for purities less than 100 percent will be discussed briefly at the end of the paper.

At this point, mention should be made of the authors whose literature has been studied during my investigation of this interesting subject. Herzfeld's (3) table of the solubility of sucrose has been the standard in the industry for more than fifty years. Other early investigators (4) have been ambitious in their attempts to challenge the validity of Herzfeld's table, but have not been wholly successful in this attempt. Holven (5) in 1936 pointed out the differences between Herzfeld's values and that data which he and his co-workers at C & H had compiled. Taylor (1) in 1947 published a paper which successfully established a new and more accurate set of values for the solubility of pure sucrose in water solutions. Taylor's data and Holven's data are in very close agreement, especially in the temperature ranges in which most commercial sugar boiling is performed.

Taylor's results should be acceptable by reason of the high degree of accuracy of the experimental work and because of the sound analysis of the results. While Herzfeld's solubility data and Taylor's solubility data do not disagree by a very large amount, the difference is significant.

Claasen's (6) boiling point elevation tables for aqueous solutions of sucrose likewise have served the industry as a standard since 1904. Again, Holven (5) challenged these data on the grounds that the variation in boiling point elevation due to vapor pressure changes was not considered in Claasen's works and this constituted a serious error in the approach to the problem. Therefore, at the insistence of Claasen, Holven and others Spengler (2) (7) and his associates, in 1938 performed new experiments and determined with acceptable accuracy the boiling point elevation of pure and impure sucrose solutions at various concentrations under numerous conditions of vapor pressure. Spengler's (7) boiling point elevation data for pure sucrose solutions are, therefore, accepted as being more correct than any other data.

Kukharenko (8) (4) in 1935 calculated a table of supersaturation and crystallization velocities for pure sucrose in water solutions. These calculations were based on Herzfeld's data for the solubility of pure sucrose in water.

Holven (9) in 1942 presented a paper in which he offered for the first time a method for determining the supersaturation of pure sucrose

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[1] Assistant Chief Engineer, The Amalgamated Sugar Company, Ogden, Utah.
[2] Numbers in parentheses refer to literature cited.

solutions. This method consisted of measuring the boiling point elevation of the sucrose solution above the temperature of the vapor phase. By resorting to the use of special Duhring line charts and formulae, he was, thus, able to determine the supersaturation of sucrose solutions.

Using this concept, Holven (10) had designed and patented in 1938 an instrument for the continuous measurement and indication of supersaturation. At that time, 1938, he found it necessary to assume, for practical purposes all Duhring lines for sucrose solutions were straight lines. This was necessary in order to construct a workable instrument. The error as a result of the above simplification in assuming straight Duhring lines as compared to the actual curved lines is not great. Gillett (11) in his book on "Low Grade Sugar Crystallization" adheres to the original concept as established by Holven. Webre (12) in his many writings uses Gillett's book as a basis for his work.

In 1954 Miller (13) presented a paper describing his company's (Minneapolis-Honeywell Regulator Company) adaptation of the Holven patents to their design of Supersaturation Meter. By 1954 instrument design techniques were such that it was possible for the designers to permit the concept of curved Duhring lines to enter their thinking. Also, in order to bring their basic data up to date this group also used Taylor's solubility data and Spengler's boiling point elevation data. It may be interesting to note that Holven's instrument and the Minneapolis-Honeywell instrument both have facilities for making a purity adjustment during the boiling of the strike.

With the preceding brief discussions and history of the subject of solubility, and supersaturation of pure sucrose solutions as a background, I would like to proceed at once to the main topic of my paper.

First, we shall take Taylor's (1) equation for "The Solubility at High Temperatures of Pure Sucrose in Water" and calculate the sucrose concentrations at saturation 1.00 for solution temperatures from 30° C. to 100° C. Taylor's work was done in the temperature range from 64° C. to 82° C. However, as pointed out in Taylor's paper others have confirmed the validity of the equation at the low temperature of 25° C. and with but few misgivings I have also proceeded to calculate concentrations at saturations for temperatures of 90° C. and 100° C.

Taylor's equation for the solubility curve of sucrose is, $C = 63.608 + 0.1322t + 0.000722t^2$, where C is the concentration in grams per 100 grams of solution, or degrees Brix, and t is the saturation temperature, degrees Centigrade. Concentration can also be expressed as S, where $S = \frac{s}{w} = \frac{C}{100-C}$, where $\frac{s}{w}$ is the ratio of sucrose to water and C is the concentration of sucrose in solution as derived from Taylor's formula. Table 1 shows C the concentration, and S the ratio of sucrose to water. These data are also displayed on Chart A in the form of S vs. t. S is plotted to a logarithmic scale and t, solution temperature, is plotted to a linear scale. An auxiliary scale is plotted along the right margin of the chart and is in equivalent values of C, or degrees Brix.

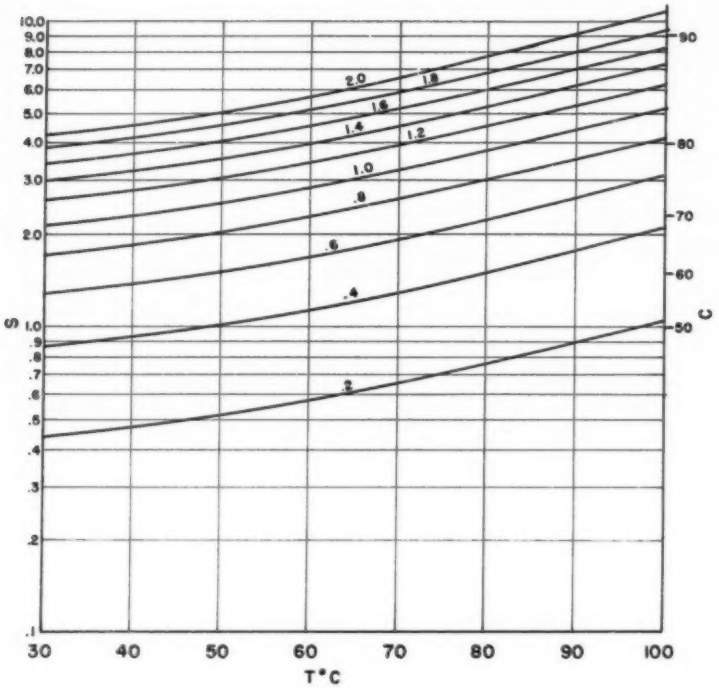

Chart A.—Saturation curves for pure sucrose.

Table 1.—Saturation Concentrations vs. Temperature at 100 Purity.

$$C = 63.608 + 0.1322t + 0.000722t^2$$

$$S = \frac{s}{w} = \frac{C}{100-C}$$

T° C.	C	S
100	84.048	5.269
90	81.354	4.363
80	78.805	3.718
70	76.400	3.237
60	74.147	2.868
50	72.023	2.574
40	70.051	2.339
30	68.224	2.147

In addition to the saturation 1.00 line as plotted on Chart A, from Table 1, there are also lines for several supersaturation values and for several undersaturation values. These can be calculated from S for saturation 1.00 as follows: For example, at a given temperature t, S′ for supersaturation 1.60 would be 1.60 x S; likewise S′ for undersaturation 0.60 would be

0.60 x S. Similarly, any other values of S′ for supersaturations other than 1.00 can be found by multiplying S at saturation 1.00 by the desired degree of supersaturation. When these other supersaturation lines are plotted on Chart A, they form a family of curves parallel to the saturation 1.00 line. The value of using semi-logarithmic coordinates becomes immediately apparent when one studies the complete family of curves on Chart A.

Next we shall take the "Boiling Point Elevation Equation for Pure Sucrose," by Spengler (2) and calculate the Boiling Point Elevation for various concentrations C at a vapor temperature of 100° C. Log_{10} B.P.E. $= 2.6157 \times 10^{-6}C^3 - 4.0185 \times 10^{-4}C^2 + 4.2567 \times 10^{-2}C - 1.1979$. These data are presented in Table 2.

Table 2.—B.P.E. vs. Concentration at 100 Purity.

Log_{10} B.P.E. $= 2.6157 \times 10^{-6}C^3 - 4.0185 \times 10^{-4}C^2 + 4.2567 \times 10^{-2}C - 1.1979$

$$F_t = \frac{(T_t)^2}{(T_{100})} \frac{L_{100}}{L_t}$$

Vap.	T°C	100	90	80	70	60	50	40	30
	Ft	1.000	.9363	.8755	.8180	.7630	.7108	.6607	.6135
	S				B.P.E.				
	10.00	20.70	19.38	18.16	16.93	15.79	14.71	13.68	12.70
	9.00	19.27	18.04	16.91	15.76	14.70	13.70	12.73	11.82
	8.00	17.69	16.56	15.52	14.47	13.50	12.57	11.69	10.85
	7.00	15.93	14.92	13.98	13.03	12.15	11.32	10.53	9.77
	6.00	13.98	13.09	12.27	11.44	10.67	9.94	9.24	8.58
	5.00	11.81	11.06	10.36	9.66	9.01	8.39	7.80	7.24
	4.00	9.44	8.84	8.51	7.72	7.20	6.71	6.24	5.79
	3.00	6.88	6.44	6.04	5.63	5.25	4.89	4.55	4.22
	2.00	4.25	3.98	3.73	3.48	3.24	3.02	2.81	2.61
	1.00	1.79	1.68	1.57	1.46	1.37	1.27	1.18	1.10
	0.50	0.74	0.69	0.65	0.61	0.56	0.53	0.49	0.45

In order to calculate the Boiling Point Elevation for Pure Sucrose solutions at a given vapor temperature less than 100° C. we must calculate a correction factor in the following manner:

$F_t = \left(\frac{T_t}{T_{100}}\right)^2 \frac{L_{100}}{L_t}$ where F_t is the correction factor at the given vapor temperature, T_t is the absolute vapor temperature °Kelvin for the given vapor temperature, T_{100} is the absolute vapor temperature °Kelvin equivalent to 100° C., L_{100} is the latent heat of the vapor 100° C., and L_t is the latent heat of the vapor at the given vapor temperature t. B.P.E.$_t$ $= F_t \times$ B.P.E.$_{100}$.

The correction factor F_t, and the B.P.E. data for vapor temperatures less than 100° C. are also presented in Table 2.

Chart B is a repetition of Chart A but with the addition of the B.P.E. data plotted from Table 2. A close study of Chart B will reveal that it is now possible for one to determine the B.P.E. and/or the solution temperature when one is given the vapor temperature and the degree of supersaturation of the solution. Conversely, given the vapor temperature and the solution temperature one can determine the degree of supersaturation of pure sucrose solutions.

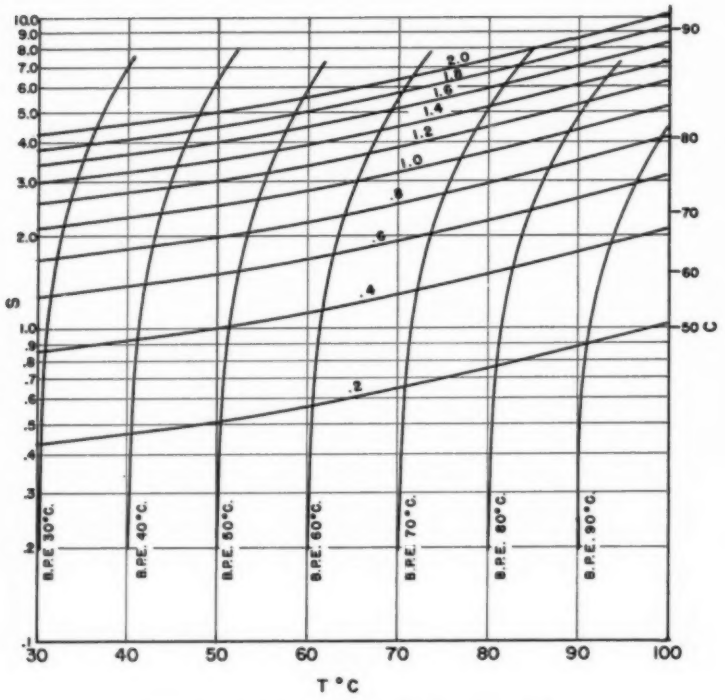

Chart B.—B.P.E. vs. saturation at 100 purity.

Table 3.—B.P.R. vs. Supersaturation at 100 Purity

Vap. T °C	50	60	65	70	80
S.S.			B.P.R.		
0.60	2.26	2.81	3.15	3.57	4.63
0.80	3.27	4.06	4.56	5.15	6.71
1.00	4.32	5.36	6.01	6.79	8.86
1.20	5.39	6.68	7.48	8.44	10.98
1.30	5.92	7.33	8.21	9.25	12.02
1.40	6.46	7.98	8.93	10.05	13.03
1.60	7.50	9.24	10.32	11.59	14.94
1.80	8.52	10.45	11.64	13.03	16.72
2.00	9.49	11.60	12.89	14.41	18.34

In order that the above data can be used readily in the sugar factory, I have calculated the exact intersections of the supersaturation lines and the B.P.E. lines for a range of vapor temperatures from 50° C. to 80° C. and for supersaturations from 0.60 to 2.00. Special emphasis is given to vapor temperature 65° C. and supersaturation 1.30. The B.P.E. values are accurate to plus or minus 0.01° C. These data are recorded in Table 3, and

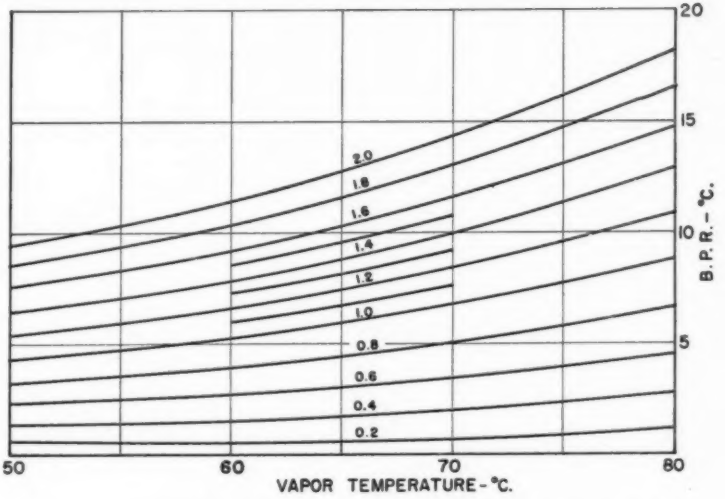

Chart C.—Saturation values at 100 purity for B.P.R. vs. vapor T.° C.

are plotted on Chart C. Note that in Table 3 and on Chart C I have changed the nomenclature for Boiling Point Elevation to Boiling Point Rise, or simply B.P.R. It appears that the latter term is more widely used than the former.

During the earlier phases of this investigation, purity correction was overly simplified; i.e., for example, at a purity of 80 percent the saturation 1.00 curve for 80 percent purity was assumed to be identical with that of the supersaturation 1.25 curve for purity 100 percent, and Spengler's B.P.E. curve for purity 80 percent was used to determine the B.P.E. values at 80 percent purity. Similar data were developed for other purities and the data were interpolated for use with the lower purity liquors in the sugar factory. We found that these data were not accurate; in fact, they were so far from being accurate that we discarded this method of correcting for purity changes.

During this campaign, 1955-56, we discovered that the B.P.R. data for white sugar boiling has varied by as much as 3° C. from the beginning of campaign to the present. This, in spite of the fact that we have endeavored to maintain a standard liquor purity within plus or minus 0.5 points of the desired purity. The only conclusion we can draw is the nature and solubility of the non-sugars has varied so greatly during the campaign as to make our former concepts of B.P.R. at saturation versus purity inadequate in explaining the differences. This has given us considerable justification for embarking on a line of study which will aid in correlating saturation, boiling point rise, purity, and the nature of the non-

sugars into a more workable form. Therefore, at this time I have chosen to confine the discussion to the one case for 100 percent pure sucrose, and to point out that considerable work must be done before we can extend this study to include the lower purity liquors.

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Hydrogen Ion Effects on the Early Growth of Sugar Beet Plants in Culture Solution

ALBERT ULRICH AND KENNETH OHKI[1]

Sugar beet plants grow well in soils of pH 6.0 to 8.0 (12)[2], but from pH 7.8 to 9.3 growth and pH have a negative correlation coefficient (3). Tomatoes and lettuce grow well in culture solutions kept at pH 5.0, 6.0, and 7.0 and Bermuda grass from pH 4 through 8.0, providing iron is supplied as a humate and all other nutrients are kept relatively constant (2). Root growth fails completely at pH 3 and top growth is greatly reduced for all plants at pH 9 (2).

Sugar beets in greenhouses make excellent growth in culture solutions supplied with iron sulfate, iron citrate or iron tartrate as a source of iron, providing the pH of the culture solution remains below 6.8 to 7.0 (17). Above pH 7.0 daily additions of iron salts are necessary to prevent iron deficiency, or alternatively, the culture solutions have to be acidified with sulfuric acid from time to time. The latter course of action is usually the simplest but even here the taking of pH readings and the addition of acid is time consuming. Care also has to be taken not to acidify the culture solutions much below pH 5.0, since at pH 4.0 injury has been observed at times. Fortunately, these precautions now appear to be unnecessary with the use of iron chelates as a source of iron.

With chelated iron in cuture solutions it has been found that sugar beet plants do not become chlorotic at a pH of 7.0, and in fact, they remain quite green at a pH of 7.4 and 7.6 (17). This observation suggested that the entire matter of pH and its effect upon the growth of sugar beet plants in culture solutions should be subjected to further experimentation. The present paper gives the findings for sugar beet plants cultured in solutions kept at a pH of 4.0, 5.0, 6.0, 7.0, 8.0 and 9.0.

Methods and Materials

The seeds of U. S. 22/3 and GW 304 A were planted on October 27, 1953. Twenty-five grams of seeds were treated with 0.25 grams of Phygon XL and planted 2.0 cm. deep in a 31 x 76 x 20 cm. germinating tray filled with vermiculite No. 2. The seedlings were watered daily with one-half strength Hoagland's culture solution No. 1 (5). When the seedlings were in the early 2-leaf stage, they were carefully removed and the roots washed nearly free of vermiculite by dipping in tap water. The individual plants were supported in a cork ring with non-absorbent cotton. Three seedlings prepared in this manner were taken at random for each 20-liter tank. Five tanks were used for a variety and pH treatment, giving a total of 60 tanks for the 2 varieties and 6 pH values. The initial composition of the nutrient solution, in millimoles per liter, was as follows: 1.0 KH_2PO_4, 2.5 KNO_3, 0.25 K_2SO_4, 1.0 $MgSO_4$, 2.5 $Ca(NO_3)_2$, 0.25 $CaCl_2$, and 0.25 Na_2SiO_3. The microelements in p.p.m. were added as follows: 0.25 B, 0.25 Mn, 0.025 Zn, 0.01 Cu, and 0.005 Mo. Iron was added one time only at the

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[2] Numbers in parentheses refer to literature cited.

rate of 4.9 p.p.m. as the ferric potassium ethylene diamine tetra-acetate complex (7). After the salts were added, the pH value of the solution was 6.7. The pH values of the solutions were adjusted to 4.0, 5.0, 6.0, 7.0, 8.0 and 9.0 with either 1.0 N H_2SO_4 or 1.0 N NaOH. All pH measurements were made with the Beckman Model M pH meter and each 20-liter tank was adjusted individually with the appropriate reagent. Measurements for pH were taken every other day for the first 16 days and daily thereafter. At these times the solutions at pH 4.0 and 5.0 tended to increase by 0.1 to 0.3 pH units, those at pH 6.0 and 7.0 by 0.2 pH units and those at pH 8.0 and 9.0 varied from 7.7 to 8.0 and 8.5 to 9.0, respectively. The culture solutions were aerated continuously starting the 10th day after transplanting. Distilled water was added during the experiment. The plants were harvested on January 5, 1955, and fresh weights of the tops and beet roots were recorded. The tops and fibrous roots were dried in a 70° C. forced draft oven and the storage roots were frozen in a 2-ounce glass jar with dry ice. The dried tops and fibrous roots were ground to pass a 40-mesh screen by means of an intermediate model, Wiley Laboratory Mill.

Potassium and sodium were determined with the Perkin Elmer Model 52A flame photometer; nitrate-nitrogen with the phenol-disulfonic acid method (9); phosphate-phosphorus soluble in 2 percent acetic acid with the phosphomolybdate method (14); and calcium with the calcium oxalate precipitation method.

Table 1.—Effects of pH on the Growth of Sugar Beets in Culture Solution.

pH culture solution	Fresh weight		Dry weight			Living leaves	Total leaves	Plant height
	Tops	Beets	Tops		Fibrous roots			
	grams	grams	grams	Percent	grams	number	number	centimeter
				pH Means[1]				
4.0	107	7.0	7.1	6.69	0.86	34.5	41.7	27.2
5.0	170	13.6	10.9	6.42	1.02	37.8	45.3	32.8
6.0	213	16.6	13.7	6.45	1.26	39.2	47.1	31.8
7.0	240	22.9	15.6	6.50	1.42	41.7	49.8	34.2
8.0	215	18.8	13.8	6.43	1.36	42.8	50.6	33.9
9.0	174	15.7	11.8	6.83	1.53	42.0	49.6	31.6
L.S.D.[2]	31	3.0	1.9	0.25	0.20	3.1	3.7	1.9
F-value[3]	18.7	25.1	19.4	3.79	13.0	8.3	6.8	14.6
				Variety Means[4]				
U.S. 22/3	177	14.8	11.6	6.55	1.15	39.9	47.9	31.0
GW 304	196	16.7	12.8	6.56	1.34	39.4	46.8	32.9
L.S.D.[2]	18	1.8	1.1	n.s.	0.12	n.s.	n.s.	1.1
F-value[5]	4.89	4.77	4.93	0.04	11.19	0.25	1.24	11.83

[1] Means of 10 values, varieties pooled.
[2] L.S.D. = least significant difference at the 5% level. n.s. = not significant.
[3] Required F-value at the 5% level is 2.43, at the 1% level, 3.46.
[4] Means of 20 values, treatments pooled.
[5] Required F-value at the 5% level is 4.06, at the 1% level, 7.24.

Table 2.—Effect of pH on the Mineral Composition of Tops, Fibrous Roots and Beet Pulp of Sugar Beet Plants.

pH culture solution	Tops[1]					Fibrous roots[1]		Beet pulp[2]	
	NO_3-N p.p.m.	PO_4-P p.p.m.	K Percent	Na Percent	Ca Percent	K Percent	Na Percent	K Percent	Na Percent
				pH Means[3]					
4.0	14,430	9440	7.52	1.83	0.67	4.26	0.08	.673	.058
5.0	18,190	4030	9.03	1.39	0.83	6.25	0.17	.724	.033
6.0	18,100	3090	9.60	0.95	0.89	7.55	0.20	.731	.024
7.0	18,290	2850	9.75	1.02	0.88	7.09	0.24	.715	.024
8.0	18,010	2550	9.79	1.14	0.67	7.16	0.27	.739	.026
9.0	16,970	2190	9.46	1.55	0.61	6.21	0.24	.756	.033
L.S.D.[4]	1,010	540	0.43	0.17	0.06	0.69	0.08	.042	.009
F-value[5]	17.9	210.3	33.5	32.1	30.9	24.3	6.5	3.6	18.2
				Variety Means[6]					
U.S. 22/3	17,270	4070	9.24	1.27	0.76	6.16	0.197	0.727	.031
GW 304	17,400	3980	9.14	1.36	0.76	6.68	0.203	0.719	.035
L.S.D.[4]	n.s.	n.s.	n.s.	n.s.	n.s.	0.40	n.s.	n.s.	n.s.
F-value[7]	0.20	0.32	0.65	3.48	<0.01	6.89	0.07	0.35	3.04

[1] Dry basis
[2] Fresh basis
[3] Means of 10 values, varieties pooled
[4] Least significant difference at the 5% level; n.s. = not significant
[5] Required F-value at the 5% level is 2.43, at the 1% level, 3.46
[6] Means of 20 values, treatments pooled.
[7] Required F-value at the 5% level is 4.06, at the 1% level, 7.24.

EXPERIMENTAL RESULTS

Effects Upon Growth

The hydrogen ion concentrations of the culture solutions had a marked effect upon the over-all growth of the sugar beet plants. At pH 4 this effect was manifested by a smaller size of the leaves, a darker green color of the leaf blades and a less vigorous development of the fibrous roots. From pH 7 to 9 there were no visible changes in the growth of the plants and neither were there any signs of necrosis or of chlorosis. Without the use of iron chelates as a source of iron, chlorosis would have developed readily at pH 7, and at pH 9 growth would have been retarded greatly.

In general, the two varieties studied, U. S. 22/3 and GW 304, responded equally to changes in pH of the culture solution even though there were significant differences in growth (Table 1) and an occasional difference in mineral composition of the plants (Table 2). When the results were analyzed statistically (4), there were no significant pH-variety interactions and for this reason the values for the two varieties have been combined in Tables 1 and 2 and in Figures 1, 2 and 3.

Tops: The fresh weights of the tops for both varieties increased rapidly from pH 4 to pH 6 (Figure 1 and Table 1). From pH 6 to 8 top growth attained a maximum value at pH 7 but none of these differences are significant at the 5 percent level (Table 1). However, from pH 8 to 9 the decrease in top weight (Figure 1) is nearly significant to the 1 percent level (Table 1).

The dry weights of the tops (Table 1) in contrast to the fresh weights, differ from each other at approximately the 5 percent level or more at each pH interval. On this basis the optimum pH for dry weight production is at pH 7 (Table 1).

The dry weight percentages of the tops (Table 1) are surprisingly uniform over the entire pH range of values especially when one considers the large differences in fresh and dry weights of the tops observed over these pH values. The lowest average value is 6.42 percent and the highest value 6.83 percent or a difference of 0.41 percentage units. While this difference is relatively small, an increase of only 0.25 and 0.33 percentage units are required for significance at the 5 percent and 1 percent levels, respectively (Student's t-test) (4). On this basis the percentage dry weight increases are significant for pH 4 and for pH 9.

Beet Roots: The storage root weights differed significantly from each other at each pH interval (Table 1). Starting at pH 4 the roots gradually increased in size until they attained a maximum value at pH 7 (Figure 1). The decline from pH 7 to 9 while small, is significant statistically (Table 1).

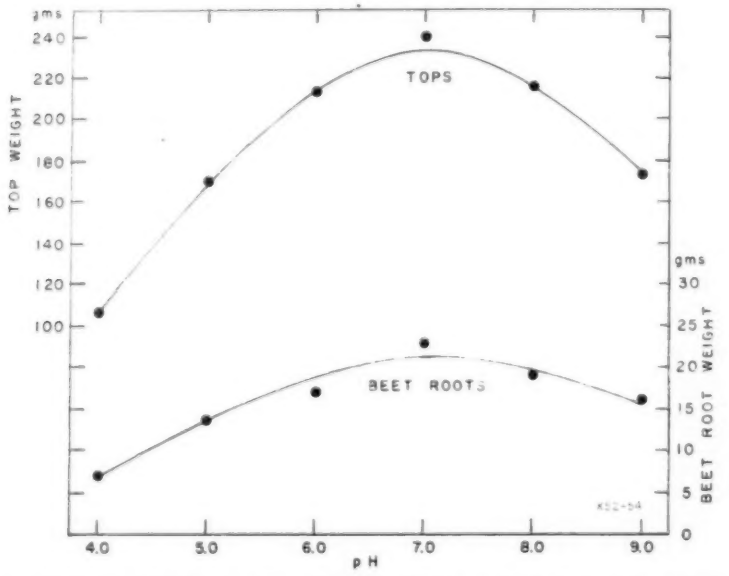

Figure 1.—Relation of top and root weight (fresh basis) of sugar beet plants to the pH of the culture solution.

Fibrous Roots: The dry weights of the fibrous roots (Table 1) increased gradually until pH 7, and thereafter, from pH 7 to 9, the pH of the culture solution had no effect upon the growth of the fibrous roots. Other than this reduction in growth, the fibrous roots remained filamentous in nature and appeared normal in all respects.

Leaf Formation and Plant Height: The total number of leaves produced by the beet plants at each hydrogen ion concentration was affected adversely only at pH 4 and 5 (Table 1). These decreases in total leaf production, while significant, were nevertheless rather small in magnitude. From pH 6 to 9, hydrogen ion concentration had no effect on the number of leaves produced by the beet plants.

The number of living leaves at the time of harvest was related to the hydrogen ion concentration of the culture solution in the same manner as the total number of leaves produced during the course of the experiment (Table 1). Apparently, leaf formation and the number of living leaves on a plant are affected less by the pH of the culture solution than the top and beet root growth.

Similarly, plant height (Table 1) is affected only slightly at the extreme pH values of the culture solutions used within this experiment. As with

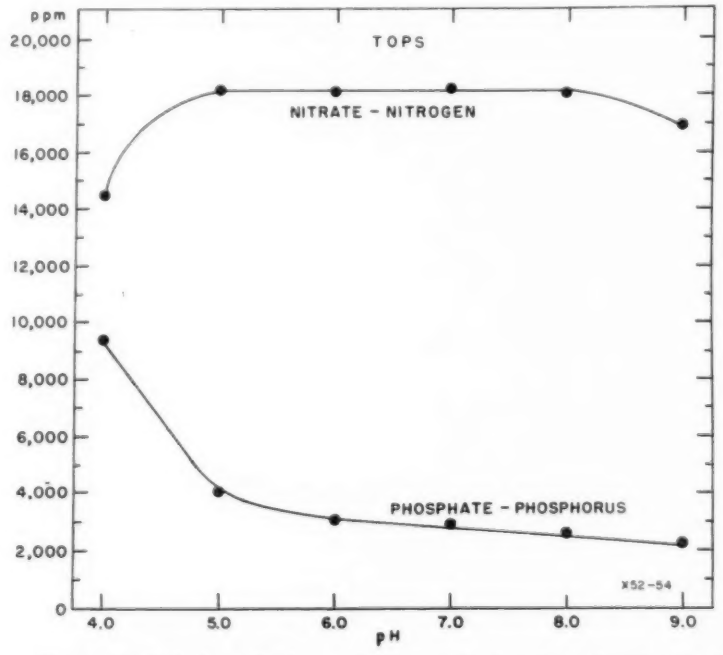

Figure 2.—Relation of nitrate-nitrogen and phosphate-phosphorus of the tops (dry basis) to the pH of the culture solution.

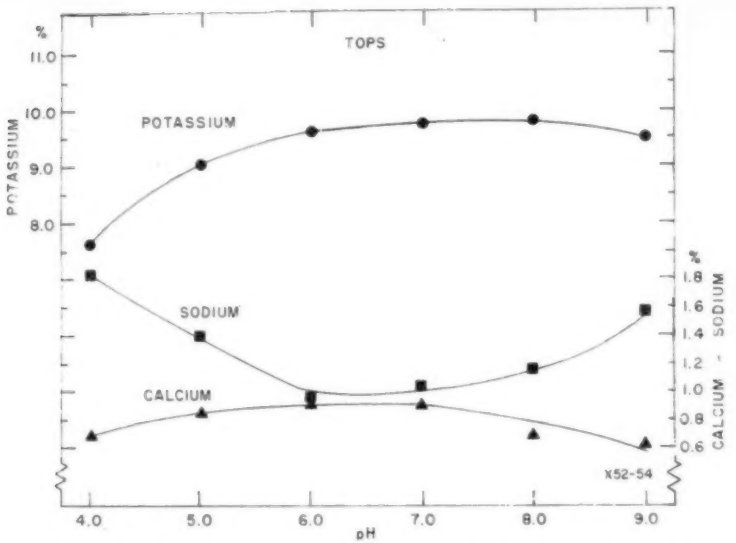

Figure 3.—Relation of potassium, sodium and calcium of tops (dry basis) to the pH of the culture solution.

the other measurements made on growth, the effects on plant height would have been greater had a wider range of hydrogen ion concentrations been used experimentally.

Effects Upon Mineral Composition

Even though a pH of 4 to 9 had no visible effect on the leaf color of the sugar beet plants, there still remained the possibility of a nutrient deficiency that could be revealed only by a chemical analysis of the leaves and the roots themselves. For example, quite often the only sign of a phosphorus deficiency in sugar beet plants is a reduction of leaf size and an intensified green color of the leaves. Under these conditions, the plants appear perfectly normal and frequently the deficiency is not detected, even by an experienced observer, unless plants with ample amounts of phosphorus are adjacent to the deficient plants. At low pH values cations, such as potassium (8, 10) and calcium (1), fail to be absorbed readily and the symptoms as ordinarily manifested at pH values from 6 to 7 could conceivably be absent at pH 4. If the hydrogen ion concentrations that cause decreases in growth are not associated with nutrient deficiencies, then the alternative possibility of a direct effect of the hydrogen ion or hydroxyl ion on growth reduction would thus appear quite likely.

Nitrate-nitrogen: The nitrate-nitrogen concentrations of the tops of the beet plants were relatively constant from pH 5 to 8 (Table 2 and Figure 2). Only at pH 4 and again at pH 9, were there significant decreases in nitrate-nitrogen concentrations but these decreases were far from indicating a

nitrogen deficiency of the plants. A deficiency would be indicated by values of 1,000 p.p.m. nitrate-nitrogen or less (15) and therefore, the cause of the growth reduction must be sought elsewhere.

As to the reason for the decrease in nitrate-nitrogen concentration of the tops at pH 4 and 9, it does not seem to be a matter of dilution either by an increase in percent dry matter of the tops or by an increased growth of the plants (Table 1). Thus, at pH 4 and 9 the grams of dry matter produced are less than at the intermediate pH values and also the dry matter percentages are relatively constant for the entire pH range study (Table 1). At pH 9 the competitive action of bicarbonate ions (8, 11) or a decreased capacity of the nitrate carrier system (6) may account for the decrease in absorption of nitrate but at pH 4 the nature of the decrease is not at all clear at present.

Phosphate-phosphorus: The phosphate-phosphorus concentrations within the tops of the plants formed an interesting pattern relative to the hydrogen ion concentration of the culture solution. At pH 4 the phosphate-phosphorus concentrations of the tops are exceedingly high (Table 2). This is followed by a precipitous decline at pH 5 and thereafter, the decreases are rather moderate in magnitude, ending at pH 9 with an average value of 2,190 p.p.m. of phosphate-phosphorus. This value is still well above the critical phosphorus value set for either petioles or blades of sugar beet plants (13) and accordingly, the differences in growth associated with pH of the culture solution cannot be directly attributable to a phosphorus deficiency within the sugar beet plant unless one is to assume that the plant requires more phosphate at pH 9 and that the phosphorus value of 2,190 is critical, whereas the value of 2,550 at pH 8 is not. At pH 4 there is the possibility that phosphate-phosphorus concentrations as high as 9,440 p.p.m. may be inhibitory to growth but as yet no definitive experiments have been run covering this situation.

Potassium: The changes in potassium content of the sugar beet plant relative to pH (Table 2 and Figure 3) offer no clue as to the cause of the decreases in growth observed at the extremes in hydrogen ion concentration of the culture solutions. Even the lowest potassium value at pH 4 is exceedingly high in comparison to the critical value of 1.0 percent potassium (16) and of course none of the values can be considered toxic because the potassium values from pH 6 to 9 are approximately identical and this includes the region of best growth. Similar conclusions can be drawn from the potassium values of the fibrous roots and of the beet pulp (Table 2).

Calcium: The calcium concentrations at pH 4, 8 and 9 decreased very little in comparison to the best plants (Table 2 and Figure 3). Since the decreases were rather small, it is unlikely that the low calcium values were associated with a calcium deficiency. Apparently, the calcium absorbed was ample to meet the needs of the plant even though the hydrogen ions of the culture solution at pH 4 may have reduced the absorption of cations and the hydroxyl ions at pH 8 and 9 tend to precipitate the calcium as calcium phosphate. Thus, it seems that the reasons for the reduction in growth associated with pH must be sought elsewhere.

Sodium: The sodium concentrations of the tops of the sugar beet plants are affected by the pH of the culture solution (Table 2 and Figure 3). Strangely enough, the highest sodium values (1.83 percent) were observed for the plants grown in solutions with the highest hydrogen ion concentration. At pH 7 to 9 the sodium concentrations of the tops increased again. Most likely these increases are the result of adding the sodium hydroxide needed to maintain the pH of the culture solution at the specified value. Sodium addition is not a factor at pH 4 and 5, where sulfuric acid was added to maintain the solutions at the specified pH values. As with potassium, the accumulation of sodium was not great enough to be toxic to the growth of the sugar beet plant.

The sodium concentrations of the pulp for each pH level are in accord with those of the tops but in the fibrous roots the concentrations follow those of potassium (Table 2).

Discussion

The hydrogen ion concentration of the culture solution has been found to have an important effect upon the growth of the sugar beet plant (Table 1). Significant reductions in top growth took place at pH 4, and contrary to expectations, at pH 5. Growth was excellent at pH 6, 7 and 8 and only at pH 9 did a significant reduction in top growth again take place.

Storage root growth for young plants differed significantly at each pH level of the culture solution. If similar effects carry over to older plants, then slight deviations from pH 7 may diminish storage root weights significantly. Experiments with larger sugar beet plants with larger storage roots would add much to our knowledge concerning the long term effects of pH upon the growth and sugar concentration of plants in culture solutions.

The chemical analysis of the tops, fibrous roots and pulp (Table 2 and Figures 2 and 3), revealed no important factor that could account for the decreases in growth observed at pH 4, 5 and 9. At pH 4 the hydrogen ions of the culture solution did not prevent the absorption of adequate amounts of potassium and calcium, and in fact, appeared to enhance the accumulation of sodium in the tops of the plants. At pH 4 phosphate accumulated to a very high concentration within the tops of the plants and it is possible that this may have interfered with the metabolism of the plant. Nitrate concentrations were reduced only slightly and could not have caused a deficiency of nitrogen in the plants. Unless further analyses reveal a specific cause of the decrease in plant growth a direct effect of the hydrogen ions at pH 4 and of the hydroxyl ions of pH 9 appears most likely to have been the cause for the decrease in growth of the plants at these pH levels.

Summary

The effect of the pH of the culture solution on the growth and mineral composition of sugar beet plants was investigated from pH 4.0 to pH 9.0 for two beet varieties, U. S. 22/3 and GW 304. Chelated iron, instead of ferrous sulfate, tartrate or citrate, was used as a source of iron.

Over the pH range studied there were no visual deficiency symptoms of iron nor of any other nutrient. In fact, the plants appeared perfectly normal except for a reduction in size of tops at pH 4, 5 and 9. Maximum top growth took place at pH 6.0, 7.0 and 8.0. Storage root growth differed significantly at all pH values, with an indicated optimum at pH 7.0. Plant height and the total number of leaves formed or living at the time of harvest were reduced mainly at pH 4.0.

Potassium, sodium, calcium, nitrate and phosphate ions were present in the tops of the plants in amounts sufficient to maintain good growth. None of these ions were below the critical level, and accordingly, the reduction in top and root growth that took place mainly at pH 4, 5 and 9 appears to be associated with a "direct" effect of hydrogen or hydroxyl ions on the metabolism of the plant rather than with an "indirect" effect upon ion absorption or upon the availability of nutrients from the culture solution.

Grants-in-aid received from the Beet Sugar Development Foundation, Fort Collins, Colorado, in support of the present study are gratefully acknowledged.

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Genetic Variability of Sugar Beets in Relation to Spider Mite Injury

H. L. Bush and H. E. Brewbaker[1]

A rather serious infestation of spider mite occurred in a field of hybrid corn adjacent to a sugar beet variety test field on the Longmont Experiment Station in 1954. The corn field was dusted with parathion and sulfur on July 28. Shortly thereafter some fading and yellowing with subsequent dying of the leaf was observed on certain varieties of sugar beets. Later on some beets were completely killed. Upon examination, this was found to be the result of feeding by mass populations of spider mites, which had apparently moved in from the corn field since the effects were first noted in beet plots adjacent to the corn.

Included in the variety test field were a number of commercial and experimental strains, topcrosses, F_1 hybrids of inbreds x red beets, and many inbred lines. As the damage from the spider mite progressed across the field, it became obvious that certain varieties, hybrids and inbreds became heavily infested and damaged while others remained remarkably free of infestation, indicating that resistance to spider mite damage is heritable. Further study revealed a rather high degree of association between known resistance to leaf spot and freedom from infestation by the spider mite. Actually, only one variety was observed to be an exception to this association —this being a curly top resistant commercial number very susceptible to leaf spot but apparently quite resistant to the spider mite.

From these observations it seems probable that the chemical, anatomical or other characteristics which make for resistance to leaf spot, perhaps also curly top, render such plants distasteful to the spider mite. Since both leaf spot and curly top were practically absent in the field, the possibility of any cause-and-effect relationship between these diseases and spider mite attack is precluded.

Figure 1 indicates the general effect of spider mite damage with a leaf spot resistant variety on the left and a leaf spot susceptible variety on the right.

Since the damage occurred as a differential effect on a variety test field, some estimate of the effect is possible as shown by the following table where a comparison is made between GW59, susceptible to the mite, and GW359 which was quite resistant. These varieties are also, respectively, susceptible and resistant to leaf spot.

	Yield of Roots		Sugar Content		Total Sugar	
	6 Loc.	Longmont	6 Loc.	Longmont	6 Loc.	Longmont
GW359	100.0	100.0	100.0	100.0	100.0	100.0
GW59	91.9	77.0	90.9	72.9	84.5	56.1

[1] Statistician-Agronomist and Director, respectively, Agricultural Experiment Station. The Great Western Sugar Company, Longmont, Colorado.

These data indicate the mean performance of the two varieties at six locations in Northern Colorado and Nebraska, where the spider mite was not a factor, as compared with their performance at Longmont where the damage was obvious. The actual figures for yield and sugar content have been calculated in percent of GW359 which was taken as 100. Although GW359 was superior to GW59 at the six locations for both yield and sugar content the difference was much greater at Longmont, with the combined effects being reflected in total sugar. GW59 produced 84.5 percent as much total sugar as GW359 for the mean of six locations, while at Longmont it produced only 56.1 percent as much. The difference between these two figures amounts to 18.4 percent and can fairly be assumed to be the result of spider mite damage.

This constitutes a first experience at this Station in which the spider mite has shown a real potential for serious injury in a field of sugar beets. Should it become a more general problem there is little doubt, judging from observations made in this field, that selection for further resistance or perhaps immunity would be effective.

The Effect of Soil Moisture and Compaction on Sugar Beet Emergence[1]

B. A. Stout, F. W. Snyder, and W. M. Carleton[2]

Farm mechanization has been responsible for increased production even though the number of farm laborers has decreased. Although considerable progress has been made toward mechanized production of sugar beets, one of the unsolved problems is that of slow, erratic emergence of seedlings. Uniform emergence is a desirable prerequisite for complete mechanization of sugar beet production.

Of the factors known to affect germination and emergence of sugar beet seedlings, soil moisture is one of the most important. Hunter and Dexter (4) reported that air-dry segmented sugar beet seeds of variety U. S. 215x216 germinated in a Brookston soil only between 12 and 20 percent soil moisture. They observed that an additional small amount of water in contact with the seed induced germination in soils drier than the critical soil moisture of 12 percent. Hunter and Erickson (5) plotted the minimum soil moisture percentages required for germination of seeds of various species in several soils on a moisture tension curve for each soil.

They found the maximum moisture tension which produced satisfactory germination was constant at 3.5 atmospheres for sugar beets, 6.6 atmospheres for soybeans, 7.9 atmospheres for rice, and 12.5 atmospheres for corn. Hunter and Erickson concluded that greater attention should be paid to the soil moisture conditions when sugar beets are planted since they require considerably more moisture for germination than other field crops.

Another factor which may have an important influence on germination and emergence of sugar beet seedlings is that of soil compaction. Because of its effect on aeration, compaction of the soil in the seed zone undoubtedly has some effect on emergence, but available references do not fully explain the effects of this factor. Hunter (3), using U. S. 215x216 segmented sugar beet seed, found that the seed would not germinate under water unless an additional supply of oxygen was bubbled through the water. Greenhouse studies conducted by Cook (2) have shown a reduction in sugar beet yields of approximately 80 percent by compacting the soil from a volume weight of 1.0 to 1.43. However, Barmington (1) stated that the planter should exert high pressures on the soil in the immediate vicinity of the seed. These and other references indicate that soil compaction and aeration are influential during germination and emergence of sugar beets. More specific information is needed in regard to the pressures which should be applied to the soil during the planting operation in order to obtain optimum emergence.

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[1] Cooperative investigations of the Michigan Agricultural Experiment Station and the Feld Crops Research Branch, Agricultural Research Service, U.S. Department of Agriculture. Approved for publication as Journal Article No. 1879, Michigan Agricultural Experiment Station, East Lansing, Michigan.

[2] Instructor in Agricultural Engineering, Michigan State University; Plant Physiologist, U.S. Dept. of Agriculture; and Professor of Agricultural Engineering, Michigan State University.

This paper presents the results of an investigation into the effect of various soil moisture conditions and compaction pressures on emergence.

Methods and Materials

Air-dry Brookston sandy loam containing 63 percent sand, 23 percent silt, and 14 percent clay was screened through a U. S. No. 16 screen. Its moisture content was adjusted to the desired level by filling a gallon container approximately one-third full of air-dry soil and adding the amount of water calculated to produce the desired moisture content. The container was closed and the mixture allowed to come to equilibrium over a period of three days during which the container was shaken for a few minutes each day. At planting time, the soil was transferred to small plastic boxes having tight fitting lids to minimize moisture loss by evaporation.

In the first experiment, soil moisture was the only variable. Approximately 340 grams of moistened soil were placed in each box, and a pressure of approximately 0.1 pound per square inch was applied to the surface of the soil. Ten U. S. 400 whole sugar beet seedballs were planted in each box by pressing each seed three-eights of an inch into the soil and covering uniformly. All plantings were replicated three times. Daily records of emergence were maintained in which only the first seedling from a seedball was recorded.

For the second and third experiments, both soil moisture and soil compaction were variables. A soil compactor[3] actuated by compressed air was used to apply the desired pressures to the soil in the plastic boxes. This device, including the four inch square aluminum foot used throughout the experiment, is shown in Figure 1. A spring scale was placed under the box to measure the force applied to the soil. The planting technique, somewhat different from that described above, was as follows: Approximately 150 grams of moistened soil were placed in a plastic box. After the soil had been leveled off, sixteen U. S. 401 whole sugar beet seedballs were placed in each box in a predetermined pattern. An additional 150 grams of soil were placed loosely over the seeds and leveled off. The box, containing the soil and seeds, was then placed under the compactor and the desired pressure applied. In experiment 2, compaction pressures of 0, 2, 5, 7, 10, and 15 pounds per square inch were applied at soil moistures of 12, 16, and 21 percent. To verify the trends indicated in experiment 2, a third experiment was designed using pressures of 2, 15, and 30 pounds per square inch at the same moisture levels. All treatments were replicated three times. After planting, the boxes were placed in a darkened room where the temperature was maintained between 65 and 75 degree Fahrenheit. Emergence counts were made daily for twelve days. When the experiments were discontinued, a soil sample was taken from each box. The moisture content was determined by oven drying and was used as a check on the moisture content determined at planting time.

[3] Designed and built by George W. French, Agricultural Engineering Research Branch, Agricultural Research Service, U.S. Dept. of Agriculture, East Lansing, Michigan.

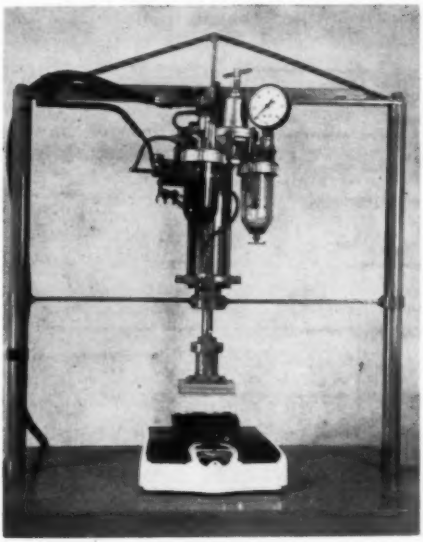

Figure 1.—Soil compactor, plastic box filled with soil, and the spring scale.

Discussion of Results

The effect of soil moisture on emergence seven days after planting is shown in Figure 2. This graph indicates an optimum range of soil moisture for sugar beet seedling emergence from 12 to 21 percent and it is in close agreement with the results of Hunter and Dexter (4). Only 3 of the 120 seedballs planted in soil having a moisture content less than

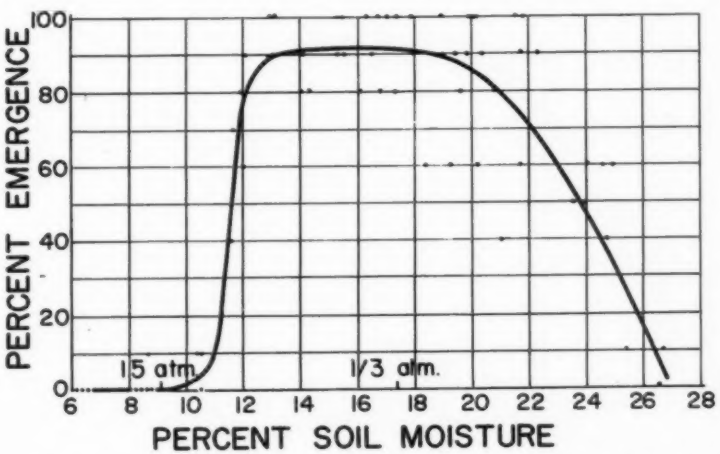

Figure 2.—Effect of soil moisture on sugar beet seedling emergence (Experiment 1).

11 percent had produced seedlings by the seventh day after planting. As the soil moisture content increased above 21 percent, there was a gradual decrease in emergence. The low percentage of emergence observed in soil having a moisture content less than 12 percent may explain, in part, the slow erratic emergence noted in the field.

Another important aspect of this problem is the rate of emergence. Rapid emergence is desirable to reduce the possibility of erratic stands resulting from surface crusting following rains. Figure 3 was plotted from the data of experiment 1. It shows the time required to reach fifty-percent emergence at various soil moisture levels. The maximum rate as well as the highest percentage of emergence occurred in soil having a moisture content ranging from 16 to 21 percent (Figures 2 and 3).

In soils with a moisture content less than the critical soil moisture required for emergence, many seeds were observed that had germinated but failed to emerge. One or two cubic centimeters of water were added to the soil surface just above a few of these seeds. These small quantities of "added" water produced marked increases in the percentage of emergence. The principle of adding water was tested in a field experiment to determine if, under dry soil conditions, emergence could be improved by adding water along the row at planting time. The results will be cited later.

The results of experiment 2, in which various static compaction pressures were applied to the soil at three moisture levels, are shown in Figure 4. Reduced emergence occurred where no compaction was applied and also where the higher compaction pressures were used. On the fourth day after planting, the highest percentage of emergence was observed in soil that was compacted with pressures of 2 and 5 pounds per square inch. After the fourth day the effect of compaction in soil having a moisture content

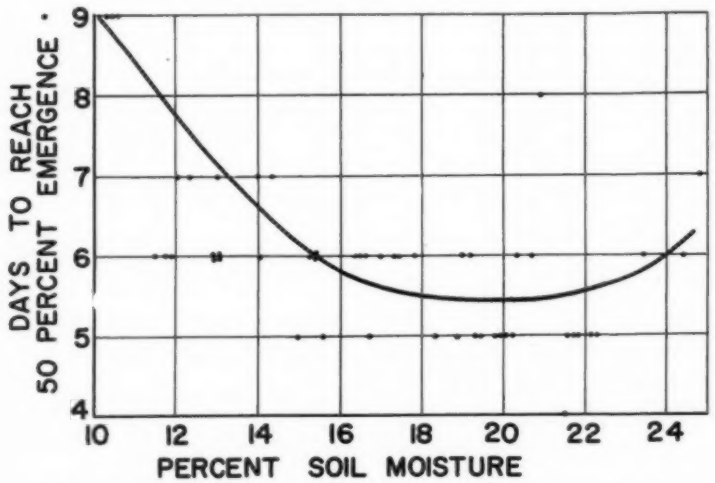

Figure 3.—Time required for sugar beets to reach fifty percent emergence (Experiment 1).

of 21 percent was no longer significant, and the emergence became somewhat erratic at all moisture levels. Possibly, after four days, the lack of oxygen and growth of micro-organisms affected emergence more than the initial compaction.

To verify the trends indicated in experiment 2, a third experiment was performed using compaction pressures up to 30 pounds per square inch. The results are shown graphically in Figure 5. In soil at 12 and 16 percent moisture a marked decrease in emergence was observed at the higher pressures. However, this effect was not apparent in soil at 21 percent moisture. At this high soil moisture level, the emergence was nearly constant over the entire range of compaction pressures from 2 to 30 pounds per square inch. Essentially the same trends are found in the data of the two compaction experiments (Figures 4 and 5).

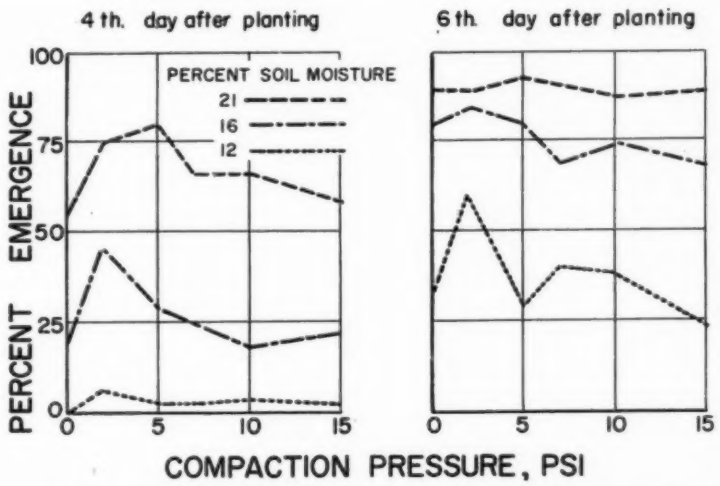

Figure 4.—Effect of soil compaction and moisture level on sugar beet seedling emergence (Experiment 2).

The depression of emergence caused by high compaction pressures at low and medium soil moistures and the absence of the effect at high soil moisture cannot be adequately explained. It seemed logical that in dry soil the higher compaction pressures would give a better seed-soil contact, thereby permitting easier transfer of moisture to the seed which, in turn, would result in faster and more uniform emergence. Also, in wet soil where moisture was not a problem, it was expected that high compaction pressures would cause decreased emergence due to poor aeration.

A field experiment was designed to further test the results found in the laboratory. Sugar beet rows were spaced 26 inches apart in Conover loam. Water was directed into the furrow just before the seed at rates equivalent to 50 and 100 gallons per acre. The planting depth was one inch. Forces

of 0, 150, and 180 pounds were applied to the soil by means of press wheels. In the first planting neither "added" water nor compaction produced any significant effect on emergence. This probably was due to rainfall totaling over five inches between planting time and the time stand counts were made. A later planting using the same rates of "added" water and forces of 0, 120, and 150 pounds on the press wheels was made under drier soil conditions. Water added at a rate of 100 gallons per acre and a force of 150 pounds on the press wheels, both produced significantly higher emergence.

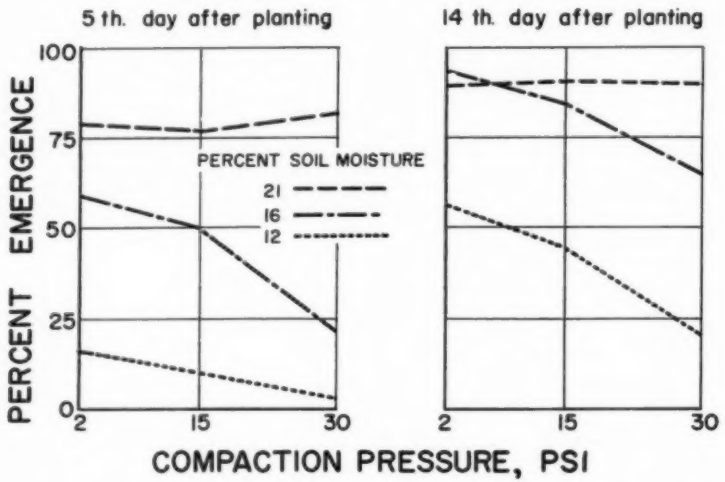

Figure 5.—Effect of soil compaction and moisture level on sugar beet seedling emergence (Experiment 3).

Summary

A laboratory study was made to determine the range of soil moisture and compaction which would produce optimum emergence of sugar beet seedlings. The results of this study were as follows:

1. Soil moistures from 12 to 21 percent produced satisfactory percentages of emergence while soil moistures from 16 to 22 percent produced highest rates of emergence.

2. In soil near the critically low moisture level, the addition of small quantities of water directly over the seeds increased the emergence considerably.

3. The optimum compaction pressure was from 2 to 5 pounds per square inch. Decreased emergence was obtained when no compaction pressure was applied, and also when pressures above five pounds per square inch were applied.

4. The effect of compaction varied with the soil moisture content. In soil at 12 and 16 percent moisture, compaction pressures above 5 pounds

per square inch reduced emergence, while in soil at 21 percent moisture and compaction pressures ranging from 2 to 30 pounds per square inch equal emergence resulted.

From a practical standpoint, this study indicates that conservation of soil moisture during seedbed preparation is of paramount importance. The use of minimum tillage practices is suggested as the most effective means of conserving soil moisture in order that the seed may be planted in soil having approximately the optimum moisture content.

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